# CORTISOL AND MALARIA IMMUNITY IN HUMAN PREGNANCY

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# CORTISOL AND MALARIA IMMUNITY IN HUMAN PREGNANCY

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ABBREVATIONS USED IN THIS THESIS

| | |
|---|---|
| ACTH | adrenocorticotropic hormone (=corticotropin) |
| ANC | antenatal clinic |
| B-cell | bursa derived lymphocyte |
| CBG | corticosteroid binding globulin (=transcortin) |
| CRF | corticotropin releasing factor |
| HCG | human chorionic gonadotropin |
| HCS | human chorionic somatotropin |
| MCHC | mother and child health clinic |
| PAM | pregnancy associated macroglobulin |
| PAS | periodic acid-Schiff reaction |
| PHA | phytohaemagglutinin |
| PDI | parasite density index |
| PPDI | positive parasite density index |
| RIA | radio immuno assay |
| SD | standard deviation |
| SEM | standard error of the mean ($SD/\sqrt{n}$) |
| T-cell | thymus derived lymphocyte |
| WHO | world health organisation |
| $X \pm SD$ | mean plus/minus standard deviation |
| $>$ | greater than |
| $<$ | smaller than |

Having been sent as medical doctor to Tropical Africa within the framework of medical services to developing countries, I was struck by the discrepancy between the wide range of tropical diseases - like malaria - with their massive effect upon the health of the population on the one hand, and the comparative lack of precise, scientifically founded knowledge about these diseases on the other hand.

Malaria, as one of the greatest public health problems of Africa, and particularly the problem of changes in immune status during pregnancy attracted my attention. I decided to engage in a study of the relationship between pregnancy and malaria, to analyse effects of malaria on mother and child, as well as effects of pregnancy on the incidence and morbidity of malaria.
Depression of immune responses to unrelated antigens as well as to malaria was demonstrated in experimental animal models. The results of experiments in the murine malaria model developed in the Department of Cytology and Histology, Faculty of Medicine at the University of Nijmegen indicate that raised corticosteroid levels are probably responsible for the lowered immunity to malaria during pregnancy.

This clinical study is the first to analyse the relation between serum cortisol and loss of malaria immunity during pregnancy, which was extensively studied in the Plasmodium berghei - mouse model. Loss of malaria immunity during pregnancy is an established feature of both the P. berghei - mouse model and human P. falciparum malaria in Africa.

From the experimental studies it was clear that in order to show the relation between serum corticoid and loss of malaria immunity during pregnancy several limiting conditions were important. Immunity in the P. berghei mouse model as well as in human malaria is of the premunition type i.e.

immune individuals are protected from disease but may carry the pathogen in small numbers. Loss of immunity may be revealed either by a recrudescence, or by a parasitaemia coming up after a reinfection (natural or after injection of parasites).
Out of the total population of immune pregnant mice those which lost immunity during pregnancy had to be selected and *compared to those which remained immune. How to make sure* that mice that did not develop a recrudescence during pregnancy did so because immunity was not lost, and not because the parasites were not present during the crucial immunosuppressive period (which is actually unknown) of pregnancy. Subinoculation of blood from the experimental animals during pregnancy into control, non-immune animals was performed to demonstrate the presence of the parasite during pregnancy. In the absence of a recrudescence only the determination of the presence of the parasite during pregnancy allowed allocation to the group of mice which remained immune during this period. Thus, when subinoculation did not reveal parasites the mouse could not be correctly allocated to any of the study groups.

In a clinical study there are, however, a number of restrictions, as illustrated by some examples. Subinoculation cannot be done, which implies that one cannot be sure that a woman who did not reveal malaria during pregnancy did so due to her immune status, and not because there were no parasites (no transmission) during the critical period (not known in humans) of pregnancy.
Furthermore, women coming to the ante-natal clinic had to receive chemoprophylactic treatment, which implies that a loss of immunity which might have occurred after this first visit may have been masked by this drug treatment.
Still another possibility is that loss of malaria immunity during pregnancy may occur as a side effect of another immunosuppressive condition e.g. another infectious disease. These restrictions hamper the correct allocation, either to the group who lost, or to the group who did not loose immu-

ity during pregnancy. In other words subgroups are inevitably contaminated with incorrectly allocated cases. It can be expected that this contamination will reduce the difference, and eventually prevents the establishment of significantly higher serum corticoid levels in cases with loss of malaria immunity during pregnancy. Thus, it is important to know as many factors as possible that affect the serum cortisol level in humans and to include them in the analysis. When an effect of age, amenorrhea or parity on the serum cortisol level is found groups have to be matched for these factors, to reduce variability. Theoretically the chance to observe the expected relation increases with the number of relevant variables included in the analyses.

An important aspect of the problem is the definition of loss of malaria immunity. Loss of immunity might be defined by an increasing number of parasites in the blood. In premune individuals the proliferation of the parasite is restricted, but the number of parasites in a premune person is unknown, and so is the level above which one has to speak of loss of immunity.
Loss of immunity might also be defined by clinical symptoms combined with parasitaemia. It is clear from both the experimental murine model as well as from human malaria that parasite positive individuals may or may not have clinical symptoms, whereas people with clinical symptoms not necessarily exhibit high parasitaemias. The better loss of immunity is defined, the higher the chance that a significant relation between serum cortisol levels will be found, the stronger the difference with the cortisol levels of the immune control group, and the more details of the relation between serum cortisol and loss of immunity during pregnancy can be successfully analysed. In this thesis "parasite positivity" as well as "clinical malaria" were used for analysis.
From the above considerations it is clear that in this study it was not possible to identify all women who lost immunity during pregnancy, and all women who did not, and to compare cortisol levels in these groups. Rather it was aimed to

identify enough factors which affect serum cortisol levels during pregnancy and to include them in the analyses. In this way it can be expected that contamination in the sub-groups with incorrectly allocated cases is limited, and will not prevent a successfull testing of the presumed relation between serum cortisol and loss of malaria immunity during pregnancy.

CHAPTER I

LITERATURE REVIEW

## A: MALARIA PREVALENCE IN RELATION TO PREGNANCY

### 1. Malaria prevalence in pregnant women compared to non-pregnant women

In a study on the effect of the pregnant state on malaria immunity we have to take into account the types of immunity to this disease (Lawson 1967). A high degree of immunity will have been acquired in an endemic malaria area due to repeated infections throughout the whole year. Malaria immunity will have been less well established in an area without malaria endemicity, or when the transmission of the malaria parasite is intermittent. In such a community epidemics can occur which affect pregnant and non-pregnant women. The effects and the resurgence of malaria in the course of pregnancy differ widely between immune women native to endemic areas and highly susceptible women, e.g. Europeans without acquired immunity.

Case reports can show the effects of malaria on pregnancy (Torpin 1941; Menon 1972; Lewis 1973), but they do not give a clear view on the influence of pregnancy on malaria immunity. Likewise, the study of malaria in parturient women (Clark 1915; Blacklock and Gordon 1925; Bruce-Chwatt 1952; Canon 1958) does not give good insight as to malaria prevalence/incidence during pregnancy and its relation to parity, age, and amenorrhea.

For the study of malaria in pregnancy as an expression of the breakdown of acquired malaria immunity (Bruce-chwatt 1952) data on the endemicity of malaria in the study area have to be considered, and malaria prevalence has to be compared in non-pregnant and pregnant women.

Reports of malaria epidemics in 1934-1935 in Ceylon which was not an endemic malaria area at that time, showed that the mortality from malaria in pregnant women was twice that

observed in non-pregnant women (Wikramasuriya 1935, 1937). Lawson (1967) stated that malaria attacks in pregnant women are more frequently seen than in non-pregnant women. Although Campbell (1980) found only a slight difference in malaria prevalence between pregnant and non-pregnant groups, he described higher parasitaemias in the pregnant women. Increased malaria prevalence and parasite density in pregnant compared to non-pregnant women has been described by Bray (1979). Comparison of data determined during pregnancy with results obtained before and after pregnancy, as well as with values obtained in a control group of non-pregnant women, showed conclusively the breakdown of malaria immunity in pregnant women (Gilles 1969; Kortmann 1972), (table I-1). Differences in malarial endemicity are probably an important factor for the different percentages reported in both studies.

Table I-1
Malaria prevalence in non-pregnant and pregnant women according to Gilles (1969) and Kortmann (1972)

| | GROUP A | | GROUP B |
|---|---|---|---|
| | before/after pregnancy | during pregnancy | non pregnant women |
| Malaria prevalence | | | |
| Gilles ; | 5.7% | 22.2% | 8.4% |
| Kortmann; | 19.0% | 41.0% | 12.0% |
| Parasite density | | | |
| Gilles ; | 140 | 1775 | 185 |
| Kortmann; | 250 | 1000 | - |

2. Malaria prevalence and parity

The loss of malaria immunity in pregnant women living in an endemic area is most marked in first pregnancies (Lawson 1967). A negative correlation between parity and parasitae-

mia has also been described (Canon 1958; Reinhardt 1978; Bray 1979).

3. Malaria prevalence during pregnancy in relation to age

Since higher birth-rank corresponds to an increased age of the mother, the mentioned negative correlation between parity and parasitaemia may be due to either of these parameters which have not been studied separately in most studies. Blacklock and Gordon (1925) did not observe any influence of age on the prevalence of parasites in the placenta. Some authors drew attention to the possible influence of age, but were not able to study these parameters separately (Canon 1958; Bray 1979). A decreasing incidence of parasitaemia in pregnant women with increasing age has been shown by Reinhardt (1978).

4. Malaria prevalence related to amenorrhea

Many studies dealing with parturient women (Clark 1915; Blacklock and Gordon 1925; Garnham 1938; Torpin 1941; Bruce-Chwatt 1952; Canon 1958; Menon 1972; Lewis 1973; Reinhardt 1978) stressed the relation between malaria and late pregnancy. A study in middle-America (Campbell 1980) did not reveal a difference in clinical malaria during the different trimesters of pregnancy. Although Kortmann (1972) did not describe a difference in his East-African study, his figures show increased parasite rates before the 25th week and after the 36th week. Gilles (1969) found that no pigment could be detected in placentas of women who received prophylactic drugs well before the 19th week as compared to women who received drugs only after the 20th week, suggesting that infections already appear during the first trimester. The highest parasite rates and densities were reported by Pingoud (1969) to occur between 16 to 28 weeks, and by Bray (1979) to occur before the 24th week of pregnancy. The latter also demonstrated lower immuno-fluorescent malarial antibodies titres (IFA) before the 24th week and concluded that the immune response to malaria parasitisation is inhibited in early pregnancy.

## 5. Malaria prevalence in the puerperal period

Although Garnham (1938) reported increased parasitaemias during the puerperal period, this finding has not been confirmed by other studies. Comprehensive studies which compared malaria prevalence in the same group of subjects during pregnancy and the subsequent puerperal period, and the malaria prevalence in these puerperal women with that of the non-pregnant women did not show any increase of malaria in the post-partum period (Kortmann 1972; Bray 1979).

## 6. Congenital malaria

Congenital malaria is malaria acquired by the unborn child from its mother owing to the failure of the barrier function of the placenta (Blacklock and Gordon 1925).

The possibility of transplacentally transmitted malaria is a well established fact. Many case reports and studies on indigenous populations of endemic malarious areas have confirmed this. Covell (1950) prepared an excellent review of the literature. A summary of the literature given by Kortmann (1972) and extended by recent publications is given in table I-2. This summary only contains literature on congenital malaria in immune populations of endemic malaria areas which have a high degree of resistance to malaria.

The incidence of congenital malaria in native populations of malaria areas, seems to be extremely low, approximately 0.03% whereas 1% to 4% was found in newborns of non-immune mothers living in hyperendemic areas (Covell 1950).

The transplacental passage of parasites is frequently described as cord-parasitaemia, but parasites usually are not found in the peripheral blood of the child. The plasmodia are apparently rapidly eliminated from the foetal circulation. Reinhardt (1978, 1980) suggested that IgG antibodies or non-specific mechanisms are responsible for this rapid elimination, but some newborns with a positive cord blood sample did not show detectable malarial antibodies in their cord blood. Reinhart (1978) suggests that the probability of congenital malaria is much higher in this situation, although Campbell (1980) could not demonstrate a protective

Table I-2; Summary of literature on the prevalence of congenital malaria

| | | | MOTHER | | | UMBILICAL-CORD | | | CHILD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | peri-pheral blood | pla-cen-ta | | | | | peripheral blood | | |
| year | author | country | n: | %pos. | %pos. | n: | no.pos. | %pos. | n: | no.pos. | %pos. |
| 1915 | Clark | Panama | 400 | 2.0 | 4.7 | 400 | 1 | 0.25 | - | - | - |
| 1925 | Blacklock & Gordon | Sierra Leone | 176 | 8.0 | 38.2 | 176 | 0 | 0 | 176 | 0 | 0 |
| 1927 | van den Branden | Congo | 55 | 56.3 | 1.8 | - | - | - | 55 | 0 | 0 |
| 1930 | Butler | Gold Coast | 328 | 23.1 | 18.0 | 50 | 1 | 2.0 | - | - | - |
| 1931 | Lombart | Congo | 50 | 56.0 | 50.0 | 50 | 1 | 2.0 | 50 | 0 | 0 |
| 1934 | Schwetz & Peel | Congo | 50 | 76.0 | 74.0 | 50 | 3 | 6.0 | 50 | 1 | 2 |
| 1936 | Daléas & Lavergne | Indo China | 887 | 1.5 | - | 887 | 3 | 0.3 | - | - | - |
| 1938 | Garnham | Kenya | 404 | 30.7 | 27.2 | - | - | - | 404 | 0 | 0 |
| 1947 | Walton | Sierra Leone | - | - | - | - | - | - | 2154 | 7 | 0.3 |
| 1948 | Peel & van Hoof | Congo | 403 | 60.2 | 37.6 | - | - | - | 393 | 0 | 0 |
| 1949 | Garnham | Kenya | - | - | - | - | - | - | 146 | 1 | 0.7 |
| 1952 | Bruce-Chwatt | Nigeria | 551 | 27.4 | 22.3 | - | - | - | 551 | 1 | 0.2 |
| 1958 | Canon | Nigeria | 117 | - | 25.6 | - | - | - | 117 | 0 | 0 |
| 1959 | Spitz | Nigeria | 576 | - | 23.6 | - | - | - | 576 | 0 | 0 |
| 1968 | Jelliffe | Uganda | 570 | 5.6 | 16.1 | - | - | - | 569 | 1 | 0.2 |
| 1972 | Kortmann | Tanzania | 962 | 23.2 | 19.7 | 1009 | 38 | 3.8 | - | - | - |
| 1978 | Reinhardt | Ivory Coast | 198 | 39.4 | 33.7 | 198 | 43 | 21.7 | - | - | - |
| 1982 | van Dongen & v.t Hof | Zambia | 155 | 8.4 | 18.1 | 155 | 9 | 5.9 | - | - | - |

effect of these passively acquired malarial antibodies during the first half year of life in El Salvador.

How the parasite succeeds in passing the placental barrier is still unknown. Neither the theory of damage to the placenta during delivery (Covell 1950) nor the hypothesis of damage by the pathological changes secondary to the infection (Wikramasurya 1935, 1937) are supported by concrete evidence. Kortmann (1972) supported the latter theory by the following two observations: first, the parasite density in maternal and placental blood films was significantly higher in deliveries with umbilical cord parasitaemias than in those without positive umbilical cord blood smears; second, the rate of contamination of cord blood with maternal erythrocytes in malaria free regions is too low to explain the cord-blood parasite rate at deliveries with positive placentas in malaria areas.

Moreover, Kortmann (1972) found more positive cord blood parasitaemia in primiparous than in multiparous women. Since the incidence of congenital malaria among non-immune women in hyperendemic regions is much higher than among immune women, and the breakdown of malaria immunity is more frequent in primiparous than in multiparous women (Lawson 1967), it may be hypothesized that the degree of breakdown of malaria immunity is related to the prevalence of congenital malaria.

## B: INFLUENCES OF MALARIA ON PREGNANCY

### 1. Anaemia of the pregnant woman

A serious effect of malaria on the health of the pregnant woman is the development of anaemia (Torpin 1941; Lawson 1967; Gilles 1969; Kortmann 1972; Menon 1972; Lewis 1973; Reinhardt 1978). Studies on anaemia of pregnant women in endemic malaria areas confirm the importance of Plasmodium falciparum infections in the aetiology of this anaemia.

Nearly all pregnant women who do not regularly receive malaria suppressive treatment are in jeopardy of anaemia compared to women who are protected by suppressive prophylactic treatment (Gilles 1969; Kortmann 1972).

Primiparous women are more prone to develop anaemia, and their anaemia becomes more severe than in multiparae (Kortmann 1972, van Dongen and van't Hof 1982). Kortmann could not explain this difference, due to lack of correlation between the parasite density and severity of the anaemia. Gilles (1969) who studied primiparous women only, could not demonstrate a relationship between parasitaemia and anaemia. His red-blood cell survival studies indicated that sometimes the onset of anaemia occurred for as late as two weeks after the disappearance of the plasmodia. Gilles concluded from these observations that the onset of anaemia was between the 16th and 24th week, while others observed onset between the 20th and 28th week (Lawson 1967). Kortmann (1972) did not observe a predilection period.

Anaemia caused by malaria is haemolytic (Gilles 1969, Kortmann 1972). The subsequently increased erythropoiesis and the need of the foetus may cause a folic acid deficiency leading to a megaloblastic anaemia. Unfortunately, other tropical diseases, such as hookworm infection, tuberculosis, schistosomiasis, diarrhoeal diseases, and deficient nutrition, affect the expected pattern of megaloblastic anaemia with symptoms of an iron-deficient anaemia.

The pathogenesis of anaemia caused by malaria in pregnant women is not completely understood. Haemolysis occurs when parasitized erythrocytes rupture at the time of segmentation

of the schizont or are removed from the circulation, e.g. by phagocytosis. Both mechanisms cannot account for the severe degree of anaemia in malaria, especially not in low grade infections.
Obviously an excessive destruction of parasitized as well as non-parasitized erythrocytes occurs (Maegraith 1948). This destruction of non-parasitized cells has been explained by an auto-immune reaction. Certain subclasses of IgG, raised against malaria antigens, react with these antigens which are expressed on parasitized as well as non-parasitized red blood cells (Facer 1980), resulting in a rapid destruction of antibody-loaded erythrocytes in spleen and liver.
In contrast to a lack of correlation between parasite density in peripheral blood and anaemia (Gilles 1969; Kortmann 1972), parasite density in the placenta correlated well with the anaemic state of the mother (Jilly 1969; Reinhardt 1978). If the excessive anaemia caused by malaria in pregnant women is considered to be an immuno-pathological process, and the placental parasite density correlates well with this anaemia, then the placenta could play a role in this immuno-pathological proces.

2. Abortion and prematurity

Pyrexia in the pregnant woman is a possible initiator of labour. Malaria associated with high fever has been related to premature labor and abortion (Torpin 1941; Lawson 1967; Menon 1972; Lewis 1973).
Although the prevalence of abortion in malarious areas has not been documented extensively, case reports (Torpin 1941; Menon 1972; Lewis 1973) suggest a causal relationship.
Since most of the African women who visit the ante-natal clinics have no record of their last menstrual period, and an accurate estimation of the pregnancy duration cannot be made from physical examination, almost every study dealing with prematurity in developing countries used the birth weight as the criterion of prematurity. A living newborn infant with a birth weight of 2500 g or less is recorded as

a premature live birth, according to the definition recommended by the World Health Organization (1950). This definition is not generally accepted for negro infants in developing countries, because their mean birth weight is lower than that of caucasian infants in developed countries, due to several factors, e.g. malnutrition, hookworm infection, malaria, and thus would contribute to a disproportionally large number of premature babies when this W.H.O. definition is used.

Nevertheless, taking a birth weight of 2500 g or less as the criterion for prematurity, malaria has been implicated as one of the causes of prematurity (Bruce-Chwatt 1952; Archibald 1956; Canon 1958; Spitz 1959). Using physical and neurological examination of the newborn according to Dubowitz (see chapter II) to assess the gestational age of the newborn, Reinhardt (1978), comparing newborn babies from malarious with those of non-malarious women, concluded that malaria plays a role in the genesis of pre-term babies.

Malaria is causally related to abortion and premature labour, but this relationship has to be analysed in more detail. Its impact on the population of pregnant women possibly correlates with the degree of malaria immunity in these women (Garnham 1938; Blacklock Mary 1941; Lawson 1967).

3. Low birth weight of the newborn

In case of parasitization of the placenta the intervillous space is usually packed with macrophages and parasitized erythrocytes. This cellular infiltration is believed to impair placental function, inducing foetal growth retardation and perinatal loss, due to antepartum and intrapartum asphyxia (Lawson 1967; Lewis 1973; Galbraith et al., 1980). The infants born to mothers with an infected placenta have a lower mean birth weight and a higher rate of low birth weight compared to children born to mothers with a placenta without malaria infection (Bruce-Chwatt 1952; Archibald 1956, 1958; Canon 1958; Spitz 1959; Mc. Laren and Ward 1962; Jeliffe 1968; Kortmann 1972; Reinhardt 1978), (table I-3).

Table I-3
Summary of African studies on the effect of placental malaria infection on birth weight.

| Study | *N: | INFECTED PLACENTAE; n: | % | difference in mean birth weight* |
|---|---|---|---|---|
| Bruce-Chwatt (Nigeria; 1952) | 310 | 73 | 23.5 | 145 g |
| Archibald (W.Nigeria; 1956) | 463 | 68 | 14.7 | 170 g |
| Archibald N.Nigeria; 1958) | 440 | 62 | 14.1 | 298 g |
| Cannon (W.Nigeria; 1958) | 392 | 130 | 33.2 | 310 g |
| Spitz (E.Nigeria;1959) | 576 | 136 | 23.6 | 89 g |
| McLaren/Ward (Tanzania; 1962) | 400 | 86 | 21.5 | 55 g |
| Jeliffe (Uganda; 1968) | 570 | 92 | 16.1 | 263 g |
| Kortmann (Tanzania; 1972) | 413 | 141 | 34.1 | 75 g |
| Reinhardt (Ivory Coast; 1978) | 196 | 66 | 33.7 | 103 g |

*N: number of women in the study.

* mean birth weight of the non-infected group minus mean birth weight of the infected group.

Other factors which have been studied in relation to low birth weight are: parity, age, maternal weight, sex of the newborn, tribe of the mother, and the socio-economic status (Jeliffe 1968; Kortmann 1972; Reinhardt 1978).
Parity is correlated to age, and both are correlated with the prevalence of parasites in the placenta; young primiparous women more frequently exhibit an infected placenta and more often give birth to children with a lower

birth weight than multiparae. Primiparous women without parasitaemia, however deliver heavier children than those with an infected placenta. In women classified according to parity and age the infants in the infected groups had a lower birth weight (Jeliffe 1968; Kortmann 1972). Tribe (Jeliffe 1968) and maternal weight (Kortmann 1972) are not correlated with the placental parasite rate, although these factors are part of the socio-economic status which in turn is correlated with the parasite rate (Reinhardt 1978); the higher the economic status the lower the parasite rate.
The effect of sex on birth weight in infected and non-infected groups is unclear. In the study of Reinhardt (1978) the correlation of parasitaemia in cord blood with lower birth weight was significant for boys but not for girls. McLaren & Ward (1962) described an opposite result, whereas Jeliffe (1968) did not find any difference in birth weight between the sexes.

## C: IMMUNITY AND PREGNANCY

### 1. Introduction

Mammalian pregnancy is an interesting immunological phenomenon of great importance, which is not yet fully explored. The embryo is a carrier of paternal histocompatibility antigens and can be considered as an allograft which is not rejected by an immune response of the mother, in spite of prolonged and intimate contact. For an overall picture of all the explanatory hypotheses which have been proposed and studied, we may refer to the very comprehensive literature reviews on this subject (Billington 1975; Lawrence 1980; Cauchi 1981). Summarizing the mechanisms which have been suggested to contribute to the survival of the foetal allograft we may mention:

a) The concept of an immunologically privileged site of the uterine environment in the same way as, for example, the anterior chamber of the eye, the testes, and the brain. The decidual tissue itself possibly has an immunologically protective role (Beer 1975);
b) the presence of an anatomical barrier, i.e. trophoblast and foetal membranes;
c) the absence of immunogenicity of the foeto-placental unit;
d) throphoblast antigens are covered by mucoproteins, immune complexes, fibrinogen, or immunoglobulines;
e) non-specific suppression of the maternal immune system which cannot react to antigenic stimuli in general;
f) local immuno-suppressive action on the surface of the syncytiotrophoblast, mediated by factors which are produced in high concentration in the placenta itself and are known immunosuppressors in high concentrations, e.g. hormones (HCS, HCG), placental proteins (macroglobulines, CBG);
g) specific unresponsiveness of the maternal immune system by immunological enhancement, expressed by blocking antibodies or immune complexes.

Most of these theories do not stand up to now established facts and can be dismissed. Studies on other theories are conflicting or not available. Current data support the hypothesis that several mechanisms cooperate to prevent rejection of the foetal allograft (Billington 1975; Lawrence et al., 1980; Loke 1978; Cauchi 1981). The following is a possible mechanism: the antigenic sites on the trophoblast are masked by certain proteins, e.g. fibrinogen, immune complexes, or immunoglobulines, preventing an efficient immune response by the mother. Moreover, the placenta produces certain hormones and proteins with immunosuppressive properties when present in high concentrations. The local high concentration at their production site in the immediate vicinity of the trophoblast may promote local immune suppression. A systemic, non-specific immunosuppression opera-

tive in the mother during pregnancy may add to this local immune suppression.

If the non-specific suppression of the maternal immune system contributes to the tolerance of the foetal allograft, it will also have implications for the response to other foreign antigens. To judge to what extent the maternal immune system is suppressed one can study the frequency and course of infectious diseases during pregnancy as well the effects of pregnancy on certain immunological disorders (Denman 1982).

Observations on human pregnancy show an increased incidence and a more severe clinical course of some viral (Waterson 1979) and some parasitological diseases (Reinhardt 1980). Studies on murine pregnancy (see below) confirm these findings and offer the possibility to investigate this unresponsiveness in relation to the humoral and cellular immune systems.

## 2. Humoral immune response in pregnancy

The antibody response in pregnancy seems to be intact (Loke 1978). There is no change (Alanen et al., 1982) or a slight increase (Gusdon 1976) in the number of B-lymphocytes (Bursa or bone-marrow derived cells) which are primarily antibody producing cells. The Rhesus-antibody production in Rhesus negative mothers and the anti-HLA-antibody production which has often been demonstrated after pregnancy, provide additional evidence. According to Lawrence et al. (1980) the mechanism of immunological enhancement exerted by production of blocking antibodies and immune complexes demands an intact humoral immune response.

The levels of immunoglobulines G, A, and M in pregnant women are comparable to those found in non-pregnant women (Gusdon 1969), although a slight decrease of immunoglobuline-G as pregnancy advances has been noted (Gilles 1969; McGregor et al., 1970; Kortmann 1972). This decrease was not always significant and has been attributed to the haemodilution which occurs during the third trimester of pregnancy (Kortmann 1972). Only immunoglobuline-D increases during

pregnancy (Gusdon and Pritchard 1972), but the reason for this is still obscure.
Moreover, an intact maternal immunoglobuline-G response is of great importance for the passively acquired trans-placental foetal immunity.

3. Maternal cell-mediated immune response in pregnancy

Observations on human and murine pregnancy revealed that an increased incidence and a more severe clinical course of viral and parasitological diseases occurred during the pregnant state.
Pregnant mice are more susceptible to several viral infections, such as "foot and mouth disease" (Campbell 1960), Coxsackie virus (Dalldorf and Gifford 1954), murine polyomyelitis (Knox 1950), intravaginal Herpes-virus type-2 (Young and Gomez 1979), encephalo-myocarditis virus (Farber and Glasgow 1968), and parasitological infections with malaria parasites (v.Zon and Eling 1980a,b), Listeria monocytogenes, and Toxoplasma gondii (Luft and Remington 1982).
In human pregnancy an increased incidence of parasitological infections, e.g. malaria (Gilles 1969,see also chapter I-A) and amoebiasis (Abioye 1972), as well as an altered course of some bacteriological infections, e.g. tuberculosis and leprosy (Lawson 1967) and the fungal infection coccidioidomycosis (Vaughan and Hall Ramirez 1951) has been claimed.
Moreover, the frequency of infections with influenza virus (Greenberg et al., 1958), hepatitis virus (D'Cruz et al., 1968), cytomegaly virus (Stagno et al., 1975; Rola-pleszczynski et al., 1977; Gerhz et al., 1981), poliomyelitis (Weinstein et al., 1951; Siegel and Greenberg 1955), varicella virus (Pickard 1968), smallpox (lawson 1967), herpes virus (Alan et al., 1970; Poste et al., 1972) and Papova virus (Coleman et al., 1977) increase during pregnancy, or the infections have a more deleterious effect on the mother.
Since intracellular viruses as well as parasites activate cell-mediated immunity (Glasgow 1970; Merigan 1974), the suppression of this part of the immune response is suspected in pregnancy.

Most studies on cellular immunity in human pregnancy concern histological and kinetic reactions of the lymphoid tissue and its constituent cells in vitro, or by experiments in vivo on pregnant rodents to avoid hazards to child or mother. This approach can only provide indirect evidence concerning the cellular response of the mother.

Most authors agree that the number of T-lymphocytes does not change during pregnancy, except during the 3rd trimester when a slight reduction tends to occur (Dodson et al., 1977; Loke 1978). However, an inversion of the B:T cell ratio during the first trimester was observed by Strelkauskas et al. (1975, 1978). Since the total number of peripheral blood lymphocytes does not significantly alter during pregnancy, this finding indicates an absolute reduction in T-cells and an increase in B-cells during the first trimester.

Nelson et al. (1973, 1977) observed the absence of germinal centers and reduced cellularity in the pelvic and para-aortic lymphnodes during pregnancy. He interpreted these observations in terms of reduced cellular immunity. Since germinal centers are B-cell areas, changes should interfere with humoral immunity. However this is not supported by current data.

In vitro studies on lymphocyte transformation to blast cells, measuring the response of lymphocytes to mitogens e.g. phytohaemagglutinin or Concanavalin-A, considered to imitate antigenic stimulation, show depression of this maternal lymphocyte response (Purtilo et al., 1972; Nelson et al., 1973; Alanen and Lassila 1982). Also the mixed-lymphocyte reaction which is an in vitro test for the ability of lymphocytes from 2 persons with different histocompatibility antigens to be stimulated, shows a depression when maternal and foetal lymphocytes are mixed (Loke 1978). Despite these findings the maternal lymphocytes can attack placental and foetal cells to kill them in vitro in the absence of autologous serum (Loke 1978; Bonnard and Lemos 1972).

Though phagocytosis is not specific, macrophages may play an important part in the effector system of immunity. Phagocytosis is claimed to be increased by some authors (Mitchell et al., 1966, 1970) or decreased by others (Nicklin and Billington 1979).

Although the intrinsic potency of all parts of the cellular immune response in the absence of pregnant serum is apparently normal, cellular immunity is obviously depressed in vivo during pregnancy.

The specific cellular response to rubella (Thong et al., 1973), cytomegaly virus (Gehrz et al., 1981), purified protein derivative (=PPD)(Smith et al., 1972; Alanen et al., 1982) are significantly reduced during human pregnancy. The curative effect of BCG(=Bacillus-Calmette-Guérin) on herpes infection diminishes also (Freedolph et al., 1974), and rejection of skin homografts in human pregnant recipients was delayed even after a second transplantation from the same donor (Andersen and Monroe 1962).

Recent studies on transplantation immunity in mice during pregnancy e.g. cardiac allografts (Baines et al., 1980), skin allografts (Smith and Powell 1977), give evidence of an immunological enhancement transferable by T-lymphocytes. In his graft-versus-host model and by tumor allograft challenge Clark et al. (1978, 1981) showed a suppression of the generation of cytotoxic lymphocytes. A non-T-suppressor-cell called suppressor null-cell is apparently involved in this process, localized in the genital tract (Clark et al., 1981). This suppression is probably mediated by serum factors, as it does not occur in the absence of autologous maternal serum and it becomes apparent before direct contact between foetal and maternal tissues takes place (Clark et al., 1981).

## 4. Immunosuppressive factors in serum during pregnancy

Humoral factors which are not present in the non-pregnant individual e.g. alpha-foeto-protein, human chorion-gonadotrophin (HCG), human chorionic-somato(mammo)trophin (HCS), and pregnancy associated proteins, are found in serum during

pregnancy. Other substances normally present in serum are increased during pregnancy, e.g. oestrogen, progesteron, and cortisol.

The immunosuppressive effect of these humoral factors can be summarized as follows:

Alpha-foeto-protein is a plasma protein produced by the foetal liver and present in high concentrations early during pregnancy (Lau and Linkins 1976). In experimental murine studies the synthesis of IgA and to a lesser extent IgG are reduced by addition of this substance. Moreover lymphocyte transformation by Conavalin-A or phytohaemagglutinin is inhibited in in vitro experiments (Murgita and Tomasi 1975 I,II; Murgita 1976) after addition of alpha-foeto-protein Sheppard et al. (1977) did not confirm these findings of a consistent immunosuppressive effect of alpha-foeto-protein in mice. In human studies alpha-foeto-protein exerted a clearly inhibitory effect on lymphocyte transformation induced by several mitogens (Lester et al., 1976; Yachnin 1976). Alpha-foeto-protein shows inter-species differences, and within one and the same species it also demonstrates certain different molecular species with different biological activity (Lester et al., 1976). It has been suggested that alpha-foeto-protein acts as a carrier for an immunosuppressive peptide or hormone (Murgita 1976).

Human chorionic gonadotropin (HCG); the immunosuppressive action of crude preparations of HCG has been demonstrated by many authors (for review see Loke 1978). Cell-mediated responses measured by in-vitro experiments were decreased and antibody responses increased after addition of HCG (Fabris et al., 1977). Purified HCG preparations did not show any effect on the immune system, indicating that the systemic immuno-depression by crude HCG is probably caused by contaminating serum protein-like factors (Morse 1968; Caldwell et al., 1975).

Human chorionic somatotropin (HCS); the function of this hormone produced by the trophoblast resembles that of prolactin and has somatotrophic hormone (STH) properties (Tausk 1976) as well.
Since non-purified preparations were used, a possible immunosuppressive effect of HCS (Contractor and Davies 1973) can be questioned by the same reason as mentioned for HCG.

Pregnancy associated proteins; macroglobulin and beta 1-glycoprotein; macro-globulin is a high molecular weight glycoprotein which is found in increasing amounts during pregnancy. The differently named glycoproteins described by several investigators (Loke 1978) probably all represent one and the same protein (Than et al., 1975, Loke 1978), which is now called Pregnancy Associated Macro-globulin; PAM. In vitro it inhibits the T-cell reactions to some extend, leaving the B-cell unaffected (Stimson 1976). PAM possibly masks antigen recognition sites on the surface of the T-lymphocyte (Cooperband et al., 1969). The immunosuppressive importance of this protein is not yet clear. The individual variation of serum levels in pregnant women is very high (4-100mg/100ml).
Moreover, PAM can sometimes be found in sera of non-pregnant females or males, as well as in the sera of patients with certain types of cancer (Stimson 1975; Loke 1978).
Beta 1-glycoprotein is a pregnancy specific protein, synthesized by the trophoblast with a possibly local immunosuppressive function at the level of the trophoblast microvilli (Horne et al., 1976).

Progesteron; results concerning the immunosuppressive role of progesteron are conflicting. Some authors (Siiteri et al., 1977) found an inhibiting effect on skin graft rejection, while others were unable to confirm this finding (Schiff et al., 1975). Specific cell mediated immunity in pregnant mice infected with

encephalo-myocarditis virus was not altered by progesteron (Farber and Glasgow 1968). Clark et al. (1981) concluded that elevated progesteron levels normally found in pregnancy did not suppress the cellular immune response, per se.
Progesteron is able to displace protein-bound cortisol from its binding sites on corticosteroid binding globulin (=CBG) (De Moor et al., 1963; Mickelson 1982). The consequence of this competition with cortisol on CBG binding sites is a possibly higher concentration of free cortisol during pregnancy. Thus, progesteron may contribute indirectly to immunosuppression during pregnancy.

Oestrogens; the results concerning the immunosuppressive effects of oestrogens are also conflicting. Farber and Glasgow (1968) described an inhibitory influence on the specific cell mediated immunity to encephalo-myocarditis virus in mice, and Ablin et al., (1974) found a reduced PHA (phytohaemagglutinin) stimulation of blood lymphocytes in mice.
Other investigators were unable to demonstrate these effects (Jenkins 1972; Schiff et al., 1975; Siiteri et al., 1977).
Like progesteron, oestradiol can displace protein-bound cortisol from the binding sites on CBG (De Moor et al., 1963). In this way oestrogen can indirectly affect the lymphoid system (see chapter I-E).

Cortisol; the gluco-corticosteroids have a well documented immunosuppressive effect on cell mediated immunity (Dougherty 1964, see chapter I-E). Levels of gluco-corticosteroids are raised during pregnancy, while cortisol, having the strongest immunosuppressive effect of all naturally occurring corticosteroids, accounts for 90% of the total corticosteroids in pregnancy (Nelson et al., 1973).

In summary, we may conclude that cell mediated immune responses are suppressed during pregnancy. The current data support a systemic immunosuppressive effect by soluble serum factors, such as alpha-foeto-protein, macro-globulin, and cortisol. The immunosuppressive effect of cortisol is well documented (see chapter I-E), whereas for the other factors there is a need for further investigations to determine the mode of action and the concentration required for active immunosuppression in human pregnancy.
The possibly indirect role of the reviewed substances and their role in relation to local immunosuppressive effects at the junction of the maternal and the foetal tissues is beyond the scope of this review which deals with the general systemic immunosuppression during pregnancy.

## D: MALARIA IMMUNITY AND PREGNANCY

### 1. Malaria immunity

**General considerations:**

Although subject of many studies, the mechanism of malarial immunity is still not fully understood (reviewed in a leading article Brit.Med.J. 1969, Wilcocks-Manson Bahr 1972, Jayawardena 1981).
Actual immunity is the sum of natural immunity and passively and actively acquired immunity.
The insusceptibility of man to infection by malaria parasites from birds or rodents is an example of innate immunity. Other innate factors are the type of haemoglobin, e.g. sickle cell trait (Allison 1954) or the preference for certain types of erythrocytes of certain species, e.g. *P. vivax* prefers young- and *P. malariae* older forms (Craik 1920).
The passively acquired immunity of the newborn is obtained by placental transmission of protective antibodies and fades after a few months (Bruce-Chwatt 1952). Subsequently the baby will suffer from malaria attacks until the recurrent infections have provided it with an actively acquired

immunity which will be established around the fifth year of life (Garnham 1949). Actively acquired immunity is provoked by the asexual erythrocytic stage of the malaria parasite, and is only directed against this stage. Immunity to malaria requires a longstanding and repeated antigenic stimulus. Withdrawal of the malaria stimulus reduces the immunity against malaria and ultimately leads to a completely susceptible host. This fading of immunity has been observed in man (Wilcocks and Manson Bahr 1976) and was demonstrated in murine malaria models (Eling 1978a,b).

The presence of the living parasite in the host is essential for the maintenance of immunity (Eling 1978b). This clinical immunity associated with continued low-grade infection is called premunition. Premunition may be considered favourable for the parasite as well as the host. It allows survival of the parasite which in turn acts as the signal for maintenance of immunity in the host. The mechanism of this balance between immunity and parasite load is not yet completely understood.Cohen et al. (1974) suggested the occurrence of antigenic variants during the course of the asexual cycle, especially in falciparum infection. On the one hand parasitaemia with the new antigenic variant is mild only due to cross-reacting antibodies, but on the other hand the parasite can survive for a long period due to this antigenic variation. These variants of surface antigens may even be induced by certain antibodies, such as those detectable by the schizont-infected cell agglutination test, the SICA test (Brown 1971). The dynamics between these antibodies and the variant-specific parasiticidal antibodies partly reflect the mechanism between parasite load and host-immunity.

The concept of antigen-antibody complexes blocking the effector mechanism of immunity and promoting survival of the parasites has been proposed by Cohen (1977).

Soluble malaria antigens are present during a malaria infection (Wilson et al. 1969). These antigens are in excess of produced antibody, thus being partly bound in immunocomplexes and partly free. This excess of soluble antigens blocks the antibody function and thus the effector mechanism of the immune response, promoting the survival of the parasites.

This last concept presumes a defect of the effector system of the immune response. Others (Eling 1980b; Jayawardena 1981) make it very plausible that the affector phase of the immune response for newly introduced antigens is suppressed as well.

**Humoral immune reponse to malaria**

The erythrocytic phase of a malaria infection strongly stimulates immunoglobulin (IgG) synthesis by inducing a polyclonal B-cell activation (Rosenberg 1978). Only a part of the IgG produced represents specific antibody against the parasite (Cohen et al., 1961). These specific malarial antibodies can be detected by serological tests (Cohen et al., 1974), but only a part of these antibodies play a role in functional immunity. Protective antibody has been demonstrated in the immunoglobulin-G class (Cohen et al., 1961, Mitchell 1976). These antibodies are directed against antigens from the merozoite coat which are normally shed during the cell-penetration of the cell by the merozoite (Bannister et al.,1975). Such antibodies are able to block the penetration of merozoites into erythrocytes and may contribute to functional immunity (Miller et al., 1975).

Auto-antibodies against red cell antigens are assumed to make the erythrocyte unsuitable for parasite penetration or growth and may contribute to the control of infection (Jayawardena 1981).

Although B-lymphocyte deficient animals exhibit more severe infections (Weinbaum et al., 1976), birds with an agammaglobulinemia after bursectomy recover from malaria (P. lophurae) infection (Longenecker et al., 1969). Transfer of immune spleen cells provide a greater protection than immune serum (Phillips 1970), and the temporary protection of this serum may depend on synergistic mobilisation of T-cell dependent responses (Jayawardena et al., 1978).

These experiments demonstrate that the humoral immune response dependent on B-lymphocytes is not clearly correlated with protective immunity. Though the B-cell response may be essential for the control of an acute infection and less

important when T-cell immunity has been developed (Jayawardena 1981), B-cell deficient mice can develop a protective immune response sufficient to terminate acute malaria in certain parasite-host combinations (Grün and Weidanz 1981).
The experiments quoted above demonstrate that the role of the humoral immune response is not yet clarified. If the immunity to malaria is mediated by multiple mechanisms (Allison and Clark 1977), the humoral response seems to be less important or even unnecessary for the development of protective immunity to certain plasmodium species.

**Cellular immune response to malaria**

Specific T-cell mediated immune responses are very important and well correlated with immunity. T-cell deficient animals suffer fatal infection, even if they were immune previously (Jayawardena et al., 1977; Eling 1979). T-cells are involved in several manifestations of malaria immunity, e.g. the monocyte response, macrophage activity, spleen response (splenomegaly), and the T-cell dependent, immunoglobulin-G antibody response (Jayawardena 1981).
Non-specific cellular responses, e.g. monocytes, macrophages and natural killer cells are increased in malaria but specific opsonisation as the only protective mechanism is unlikely (Jayawardena 1981). Recently this non-specific immune response was stressed by the hypothesis that natural killer cells and macrophages may be bound to parasitized red blood cells producing a superoxide anion ($O_2$). This oxidant stress could lead to degeneration of parasites intracellularly (Allison et al., 1982).

**Immunosuppression in malaria infection**

Immunosuppression in malaria infection is clinically evident by the greater severity of intercurrent infections, especially in children. Measles, gastro-enteritis, respiratory infections are seen more frequently during a malaria infection (Williamson et al., 1978). These clinical observations were confirmed in experimental, well defined malaria models

of rats and mice (Golenser et al., 1981; Eling 1980b). Involution of lymphatic tissue in thymus and spleen (Eling 1980b) as well as suppression of phytohaemagglutinin stimulation of lymphocytes (Golenser et al., 1981) indicate suppression of T-cell mediated responses. The immunosuppression is spleen-cell dependent (Eling 1980b,c; 1982a) and invalidates the afferent part of the immune response, preventing the development of an adequate protective immunity.
The mechanism of this suppression is not completely understood. Reduced secretion of lymphocyte activating factors and a super-normal secretion of suppressor factors by macrophages has been claimed by Wyler (1979) to be the cause of the observed immunosuppression. T-suppressor cells (Golenser et al., 1981) or T-helper cells involved in the production of blocking immunoglobulin-G (Eling 1982b) are possible explanations.
The balance between protective host responses and immunoregulatory processes, both triggered by the parasite, is clinically expressed by the concept of premunition. This balance is established precariously and can easily be disturbed by intercurrent diseases, malnutrition, pregnancy, or be manipulated in experimental models.

## 2. Malaria immunity in pregnancy

T-cell dependent cellular immune responses and, to a lesser extent, protective antibodies are assumed to play an important role in malaria immunity. During pregnancy the humoral responses appear to be normal, but the T-cell dependent immune responses are supressed (chapter I-C). Thus, pregnancy dependent depression of cell mediated immunity may affect malaria immunity. Higher prevalence of malaria in human pregnancy (chapter I-A) as well as in murine malaria (van Zon and Eling 1980a, 1980b) supports this assumption.
Some studies on titres of specific malarial antibodies showed no differences between pregnant and non-pregnant women (Gilles 1969; Kortmann 1972). Not all of these specific malarial antibodies are related to protective

immunity. In other studies, the level of immunoglobulins-G and M were found to be decreased during the 3rd trimester (Gilles 1969; Kortmann 1972; McGregor 1970), which was explained by the haemodilution effect of gestation.
Decreased levels of falciparum specific immunoglobulin-G early in pregnancy were observed by Bray and Anderson (1979). This was related to a higher prevalence and parasite density.
Since protective cell mediated malaria immunity cannot yet be assessed in human pregnancy, studies in a murine malaria model, which also shows a loss of malaria immunity in pregnant mice (van Zon and Eling 1980a,b), are important. This murine malaria model shows an increased parasite clearance early in pregnancy, followed by a period of immunodepression during which recrudescences occur (van Zon and Eling 1980a). Prevalence of malaria in human pregnancy is also related to certain gestational periods (chapter I-A).
The loss of immunity in the murine malaria model is also found in human pregnancy, where genetic diversity plays an important role in the malaria prevalence within and between communities (Lawson 1967).
Recrudescence occurred less frequently in gravida II mice than during their first pregnancy (van Zon and Eling 1980b), which is in accordance with lower recrudescence rates in multigravid relative to primigravid women. One factor may be the age-dependent reinforcement of immunity. An improved malaria immunity in older women has been suggested by Canon (1958; chapter I-A). In murine malaria the reduced period of premunition before conversion to sterile immunity in older mice might indicate an improvement of malaria immunity with age (Eling 1980a).
Other studies suggested that the confrontation of the parasite with the immune system during gestation reinforced existing, or induced additional, immune reactions which are not suppressed during a subsequent pregnancy (Eling 1982b; van Zon et al., 1984).
Persisting parasites in an immune non-pregnant host do not trigger this reaction, and the suppression of cell-mediated

immunity in pregnancy is probably essential for the induction of this additional immune response.

## E: CORTICOSTEROIDS AND PREGNANCY

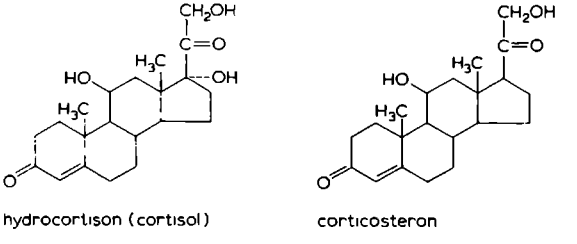
### 1. Corticosteroids and the immune system

**General considerations:**

The gluco-corticosteroid hormones produced by the cortex of the adrenals have been studied extensively. Their regulating or modulating function in many physiological systems as well as the pathology associated with either deficiency (Addison's disease), or increased levels (Cushing's syndrome) have been reviewed repeatedly (Bach 1975; Baxter and Harris 1975; Claman 1975; Tausk 1976).

In this chapter some immunoregulatory properties of the gluco-corticosteroids are mentioned which may be relevant for the investigations described in this thesis. No attempt has been made to summarize all available information.

The most important adrenocortical hormone in man is cortisol (hydrocortison or Kendall's compound F). It is the most active biological component of all corticosteroids produced in man (Dougherty 1964). Cortison, another quantitatively important corticosteroid in humans, is partly metabolized to cortisol, although it has its own biological activity as well (fig. I-1), (Tausk 1976).




hydrocortison (cortisol) corticosteron

Fig. I-1; Hydrocortisone and corticosterone

The daily production of cortisol in man is 15-30 mg, the biggest fraction of which is bound to a plasma alpha-globulin, transcortin (CBG= Corticosteroid Binding Globulin), (for review see Brien 1981). The unbound cortisol fraction in plasma is considered to be related to the biological activity of the corticosteroids in its different effects (Slaunwhite et al., 1962). The secretion and production of corticosteroids in the adrenal cortex is regulated by the adrenocorticotropic hormone (ACTH). ACTH is secreted by the pituitary gland. This secretion is stimulated by a cortisol releasing factor (CRF) from nuclei in the hypothalamus (Tausk 1976), which in turn can be inhibited by the free fraction of cortisol in plasma: the negative feed-back system (Kawai and Yates 1966). The secretion of CRF, ACTH, and thus of cortisol, shows a diurnal rhythm with the lowest secretion at mid-night and a peak level in the early morning hours. This diurnal rhythm is obscured when high concentrations of cortisol are present, as in Cushing's syndrome (Tausk 1976), although a subset of patients with Cushing's disease demonstrated different cortisol levels at various times of the day (Pieters 1982). This circadian rhythm is still present during gestation, although the day-night variation is reduced (vide infra). Studies on the effects of both cortisol deficiency, e.g. congenital adrenogenital syndrome and increased plasma levels, e.g. intoxication, prolonged supra-physiologically dosed corticosteroid medication and Cushing's syndrome have revealed the importance of this corticosteroid. For most of these effects we may refer to the textbooks. Here we concentrate on the suppressive effects which cortisol may exert on certain parts of the immune system.

**The immunosuppressive effect of gluco-corticosteroids:**

The immunosuppressive properties of gluco-corticosteroids exhibit species specific differences (Claman 1972; Block et al., 1982). Administration of gluco-corticosteroids causes a severe, dose dependent involution of the thymus and other lymphatic organs (Dougherty 1964), by lymphocytolysis in

rodents (Dougherty 1964; Adolf and Swetly 1979), which cannot be provoked even by high doses in man (Fauci 1974). Administration of cortisol produces a transient neutrophyllic leucocytosis, lymphocytopenia, eosinopenia and monocytopenia for a few hours only in humans (Fauci 1974; Block et al., 1982). This lymphopenia has been explained by a redistribution of cells rather than lysis of cells (Andersen et al., 1969). Thomson et al. (1980) suggested that circadian variation of the plasma cortisol levels regulated the circadian variation of the amount of peripheral blood lymphocytes, indicating that redistribution of lymphocytes between blood and extravascular compartments is affected by minor changes in serum cortisol levels in man.

Sensitivity to the immunosuppressive properties of glucocorticosteroids not only differs between species, but also between lymphocytes of one species (Fauci 1976), depending on the developmental stages of each cell (Claman 1972;Galili et al., 1980).

Moreover, B-lymphocytes are more resistant to the immunosuppressive action of gluco-corticosteroids than T-lymphocytes (Fauci 1974; Block et al., 1982). This resistance has been explained by a higher metabolism of cortisol by B-lymphocytes, which protects them against this hormone (Klein et al., 1980). As a consequence of this difference in sensitivity to gluco-corticosteroids, humoral immune responses are less suppressed than cellular responses (McMillan et al., 1976; Block et al., 1982). McMillan (1976, 1979) found depressed IgG synthesis by B-lymphocytes in bone marrow, whereas B-lymphocytes in peripheral lymphoid tissue showed enhanced specific antibody production after corticoid treatment (Cooper et al., 1979).

The suppression of specifically activated T-cells by glucocorticosteroids results in suppression of cell mediated immunity (Galili et al., 1980). Suppression of the T-cell dependent proliferation of macrophages as an important effect, in cell-mediated immune reaction has been variously explained by reduced lymphokine production (Duncan et al.,1982) or inhibition of lymphokine interaction with macrophages (Block et al., 1982).

In summary the cellular immune response seems to be more suppressed by increasing gluco-corticosteroid concentrations than humoral immune responses, though the results of different studies on the site and mode of action of the immunosuppression are still incomplete.

## 2. Corticosteroid-binding-globulin (CBG, transcortin) during pregnancy

CBG plays an important role in regulating the amount of free cortisol either directly or indirectly through binding of cortisol.

Only 5 to 10% of serum cortisol is not bound to protein in plasma and is generally assumed to be metabolically active. Most plasma cortisol is bound to protein, either to CBG (approx. 70%) with its high affinity or to albumen (approx. 20%) with its low affinity for cortisol. The maximum binding capacity for cortisol in normal human plasma is about 20 to 25 g cortisol per 100 ml plasma.

One CBG molecule can bind one cortisol molecule. Other steroids, e.g. desoxycorticosteron, 11-desoxycortisol, progesteron, and 17-OH-progesteron compete with cortisol for the binding sites on CBG (table I-4), (De Moor et al., 1963;

Table I-4
Influence of various steroids in vitro on the transcortin binding of cortisol

| STEROID | cortisol displaced |
|---|---|
| dexoxycortisol | 11% |
| progesteron | 23% |
| 11-desoxycortisol | 36% |
| 17-α-OH-progesteron | 46% |

(adapted from De Moor et al., 1963)
50% displacement indicates an equal ability to bind to CBG

Mickelson 1982). The probably important regulatory functions of this competition of binding is indicated by the considerable affinity of progesteron for CBG in combination with increased levels during pregnancy.

CBG is normally produced in the liver, and this production can be stimulated by oestrogens. This oestrogen dependent CBG production in the liver is limited, since CBG levels in oestrogen treated persons do not surpass a twofold increase. This maximum production is induced by a certain concentration of oestrogen, above which no further increase in CBG levels is measured (Katz and Kappas 1967).

CBG production in the liver is also stimulated during pregnancy by oestrogens in a direct dose dependent manner from the 9th week of gestation onwards, when oestradiol and oestron have surpassed a certain threshold (Moore et al., 1978). The level of liver CBG increases during pregnancy from non-pregnant values at 8 weeks of gestation to a maximum level obtained after 28 to 32 weeks of gestation and remains high until the third day after delivery, when a normal, non-pregnant value of CBG is found (De Moor et al., 1966; Wilson et al., 1979).

The additional increase in plasma CBG during pregnancy is brought about by an oestrogen induced synthesis of placental CBG in the syncytiotrophoblast (Werthamer et al., 1976).

Since unbound cortisol is rapidly excreted by the kidneys, an important role for CBG is to prevent renal loss and to reduce the rate of cortisol synthesis by the adrenals. The CBG-cortisol complex however, is apparently not directly involved in the negative feed-back mechanism between the adrenals and the hypothalamus (Kawai and Yates 1966), nor in the stimulation of glycogen synthesis in the liver, which depends mainly on free cortisol (Slaunwhite et al., 1962). Other effects, such as increased activity of tyrosin-aminotransferase in the liver, or a decrease in lymphocytes in the peripheral blood can equally well be induced by bound and unbound cortisol (Rosner 1972). Further evidence for a regulatory role of bound cortisol, or CBG alone was suggested by the finding that both substances can be incor-

porated by lymphocytes where they interfere with protein synthesis (Amaral 1971; Werthamer et al.,1971, 1973).
Moreover, Amaral and Werthamer (1976) and Werthamer et al. (1976) described an inhibitory role of transcortin on cell-mediated immune functions.
The CBG of placental origin is slightly modified compared to liver CBG and exerts an even greater immunosuppression than the latter (Werthamer et al., 1976). Rosner (1982) claims, that it is not CBG itself, but the cortisol-CBG complex, which is responsible for this suppression.
Summarizing the data so far we may conclude that CBG does not only prevent rapid excretion of free cortisol by the kidneys, but the CBG-cortisol complex also has an immunoregulatory role which may be of particular importance during pregnancy, when CBG-cortisol complex levels are substantially increased.

## 3. Corticosteroids during human pregnancy

**ACTH in pregnancy**

The absolute values of ACTH concentrations in pregnancy reported in the literature vary considerably, probably because of methodological differences. Carr (1981) measured ACTH during gestation, labour, and during the post-partum period longitudinally in the same women. He concluded that ACTH levels are significantly lower throughout pregnancy compared to non-pregnant women but ACTH levels are more suppressed in early pregnancy than in late pregnancy. This rise in ACTH occurs in spite of higher cortisol, oestrogen, and progesteron levels which are known to reduce ACTH secretion (Vale et al., 1978).
The presence of an ACTH-like substance of placental origin has become evident (Ross et al., 1975; Genazzani et al., 1975; Liotta et al., 1977). This ACTH-like substance is already present early in pregnancy at the 7th to 10th week, and its level remains stable till the 37th week, when it decreases slightly (Genazzani et al., 1975, Liotta et al., 1977). Its placental production is not depressed by dexame-

thason (Liotta et al., 1977), which suggests that the cortisol production in the adrenal glands can be stimulated in the absence of a negative feed-back mechanism for placental ACTH. Moreover, longitudinal studies during gestation revealed an increasing responsiveness of the maternal glands to ACTH, which resulted in higher levels of total and free cortisol (Nolten and Rueckert 1981).

**The circadian rhythm of cortisol levels**

The ratio of evening and morning levels of total cortisol is lower in pregnant women compared to non-pregnant women. (Burke et al., 1970; Lindholm 1973). Carr (1981) measured total cortisol and ACTH levels in the same women during 24 hours in all trimesters of pregnancy and concluded that the diurnal variations are quantatively similar to those seen in non-pregnant women. These findings were confirmed by the results of the study of Cousin (1983), but this author found a significant blunting of the proportional deviation of the 24-hour mean of plasma cortisol with advancing gestation. Nolten and Rueckert (1981) compared the free cortisol levels of the same women during pregnancy and post-partum at 20 minute intervals during a 24 hour period. They found a normal circadian pattern in the pregnant women, in spite of the elevated cortisol levels and an increasing resistance to dexamethason suppression of ACTH production. The circadian changes in the ACTH secretion pattern of pregnant women suggests that the circadian fluctuation of cortisol is regulated by normal mechanisms which would be expected to be attenuated or abrogated by the placental source of ACTH-like substances.

Nolten and Rueckert (1981) suggested that the maternal hypothalamo-pituitary feedback mechanism is reset at a higher level during pregnancy. The suggested reduced sensitivity of the hypothalamo-pituitary axis could be explained by high maternal plasma concentrations of progesteron and oestradiol which antagonize ACTH secretion (Vale and Rivier 1978; Jones 1978; Demey-Ponsart et al., 1982). A hypothalamo-pituitary reduced sensitivity for the negative feed-back by free

cortisol is needed to maintain a normal, though blunted circadian rhytm and to react appropriately to physical and psychological stress. The condition of a constant high level of bound and unbound cortisol is maintained by the autonomously working source of placental ACTH, and the pituitary ACTH induced cortisol fluctuation is superimposed on this.

**Total cortisol levels during pregnancy**

The concentration of cortisol, measured as the total amount of bound and unbound cortisol fractions in plasma, is increased during gestation (Bayliss et al., 1955; Burke 1958; Doe et al., 1960; Bro Rasmussen et al., 1962; Burke et al., 1970; Genazzani et al., 1975; Carr et al., 1981; Demey-Ponsart et al., 1982; Cousins et al., 1983). The absolute cortisol values in plasma measured by these investigators vary substantially, probably due to differences in determination procedures, as was already mentioned for ACTH.

All authors recorded an early increase around the 7th to 10th week of gestation. Some authors suggested a progressive rise till the end of the second trimester (26th to 32th weeks) remaining constant until labour commences (Carr et al., 1981; Demey-Ponsart et al., 1982).

At delivery cortisol values increase tremendously but return to normal non-pregnant levels three days after parturition (vide infra).

**Free cortisol levels during pregnancy**

An increased excretion of free cortisol by the kidneys during pregnancy (Lindholm and Schultz-Möller 1973), which is a function of the plasma non-protein bound cortisol concentration (Lindholm 1973) points to an elevated level of free cortisol (Lindholm and Schultz-Möller 1973). Direct evidence for an elevated concentration of free cortisol is given by the studies of Doe et al. (1960, 1969), Burke et al. (1970), Ress et al. (1975), Nolten and Rueckert (1981), Demey-Ponsart (1982) and Cousins (1983).

The increase of the free cortisol fraction in the study of

Nolten and Rueckert (1981) is remarkably higher compared to the findings in the other studies mentioned. They are the only authors who report the birth rank; all their women were primigravidae.

**Oestrogens and cortisol**

Plasma oestrogen levels increase during gestation as soon as nidation takes place. Around the 9th week of gestation oestradiol and oestron surpass a threshold (Moore 1978) for the stimulation of CBG synthesis in the liver (Sandberg 1959; Mills et al., 1960; Doe et al., 1960; Peterson et al., 1960; Katz and Kappas 1967). Increased synthesis of CBG by oestrogens in pregnancy is followed by an increased level of total cortisol in plasma (Sandberg and Slaunwhite 1959),as in oestrogen treated subjects (Doe et al., 1960; Mills et al., 1960; Petersom et al., 1960; Burke et al., 1969). When more CBG is available in the plasma, more unbound cortisol is bound to its carrier protein leading to a lower level of free cortisol which in turn affects the negative feed-back mechanism of the hypothalamo-pituitary-adrenal axis, leading to an increased release of cortisol by the adrenals.

The increase of cortisol in oestrogen treated subjects was shown to be due to a rise in the protein bound fraction, only, by Mills et al. (1960) and Doe et al. (1960), but other authors also found an increase in the non-protein bound cortisol (Player et al., 1964; Burke et al., 1969, Doe et al., 1969; Lindholm 1973).

The influence of oestrogens on the plasma cortisol concentration as a result of raised CBG level is not the only possible explanatory mechanism for the raised cortisol level. The biological half life of cortisol in oestrogen treated subjects is increased, whereas the daily secretion rate is reduced (Peterson et al., 1960; Layne et al., 1962; Sandberg et al., 1967). The excretion of cortison, dehydro- and tetrahydro-metabolites of cortisol are decreased (Peterson et al., 1960; Layne et al., 1962). Brien (1981) suggests that the enzyme activity involved in catabolism of cortisol in the liver may be decreased by oestrogens.

**Cortisol levels during labour and the post-partum period**

Stress situations in man stimulate the hypothalamo-pituitary function, causing a higher cortisol concentration. If we consider labour a stress condition which stimulates ACTH release concomitant with higher cortisol production, we have to accept the hypothesis of a normal hypothalamo-pituitary axis during pregnancy.

Indeed, plasma levels of ACTH rise during labour and drop quickly thereafter (Kauppila et al., 1974; Carr et al., 1981). Together with this rise of ACTH an increase of cortisol concentration is found (Gemzell 1954; Bro-Rasmussen et al., 1962; Kauppila et al., 1974; Okada 1974; Predine et al., 1979; Carr et al., 1981), which also correlated with the degree of cervical dilatation (Kauppila et al., 1974). Primigravidae appear to have higher levels of 17-OH-corticosteroids than multiparae. This difference was also reflected in the venous cord-plasma levels of corticosteroids (Gemzell 1954). This corticosteroid appeared to be cortison, since most cortisol is only transformed into cortison in the placenta before being transferred to the foetus (Murphy et al., 1974).

The cortisol levels drop quickly after delivery and reach the normal non-pregnant level within one week (Bro-Rasmussen et al., 1962). Free cortisol in serum returns to normal values within 1 to 4 days, but total cortisol does not change during this period (Demey-Ponsart et al., 1982). They explained the observation by the longer half life of CBG (6 days). The binding sites on CBG become occupied by free cortisol after the delivery, replacing the placental steroids, the levels of which drop immediately after uterine-placental separation.

## F: REGULATOR FUNCTION OF CORTICOSTEROIDS IN MURINE MALARIA

Pregnant as compared to non-pregnant women exhibit an increased incidence of malaria and higher parasite densities.

In the experimental Plasmodium berghei murine model, as in human malaria, reduced responsiveness and even complete loss of malarial immunity were observed during pregnancy.
The generally described reduction in cell mediated immunity during pregnancy and the T-cell dependency of malarial immunity suggested that increased prevalence of malaria during pregnancy was caused by a pregnancy associated suppression of T-cell mediated immunity.
Since serum levels of cortisol (man) and corticosterone (mice) increase during pregnancy and these corticosteroids are known to suppress T-cell dependent immune responses, they were considered a possible regulator of immune function during pregnancy. This hypothesis was studied in the Plasmodium berghei murine model (van Zon et al., 1982, 1983).
An increase in the serum level of corticosterone (the physiologically important gluco-corticosteroid of mice) during pregnancy was found to be correlated with thymus involution, which may be a marker for suppression of T-cell dependent immune responses (Eling et al., 1977).
Thymus involution during pregnancy was prevented by adrenalectomy, except at the end of pregnancy when the corticosterone level increased due to production by the foeto-placental unit (Barlow et al., 1974). In addition, increased levels of plasma corticosterone correlated with loss of malarial immunity during pregnancy (van Zon et al., 1982; fig.I-2), and a strongly reduced recrudescence rate was observed in mice adrenalectomized before pregnancy (fig.I-3). Moreover, adrenalectomized mice with recrudescence during pregnancy exhibited comparatively high corticosterone levels (of foeto-placental origin) during the last period of pregnancy (van Zon et al., 1982).
The average corticosterone concentration as well as the magnitude of the standard deviation in mice developing recrudescences later during pregnancy shifted from the value found in the group without recrudescence to that in the group actually experiencing a recrudescence. This growing significance in the increase of serum coricosterone

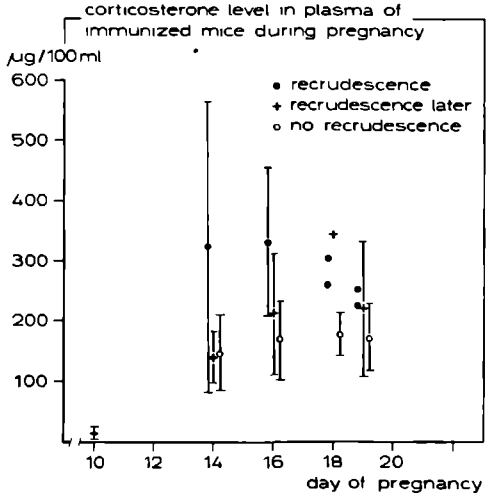



Fig. I-2; Plasma corticosterone concentrations during pregnancy Of 37 mice, 24 (65%) developed recrudescence. Plotted corticosterone levels are mean values (± standard deviation) of each group of mice. On day 10 of pregnancy the results were pooled since corticosterone levels of the groups were the same. Symbols: •, mice actually having recrudescence, +, mice developing recrudescence later in pregnancy; o, mice without recrudescence throughout pregnancy.
(From v. Zon et al., 1982)

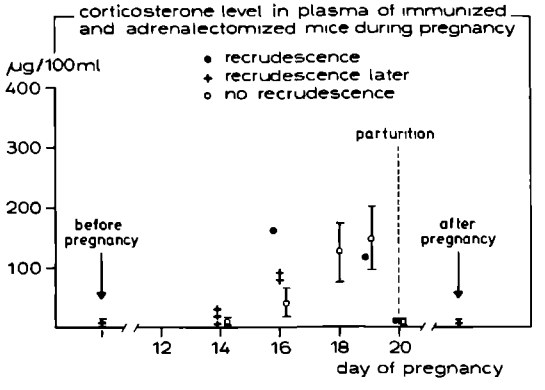



Fig. I-3; Plasma corticosterone concentrations in adrenalectomized pregnant mice (n=22) Plotted values are the mean (± standard deviation) of mice without recrudescence, whereas values of recrudescent mice are plotted individually. Symbols: •, mice actually having recrudescence; +, mice developing recrudescence later in pregnancy; o, mice without recrudescence throughout pregnancy.
(From v. Zon et al., 1982)

concentration towards recrudescence could also suggest a (direct) relation between serum corticosterone levels and parasitaemia (van Zon et al., 1982).
It is important to note that infection per sé did not result in comparable increases in total serum corticosterone levels (van Zon et al., 1982).
As for total serum corticosterone, there was a correlation between loss of malaria immunity during pregnancy and increased levels of free corticosterone. Free corticosterone was also increased , however, in infected non-pregnant mice after 1 week of infection, despite more or less stable levels of total corticosterone (van Zon et al., 1982). Likewise, free levels extensively increased further in recrudescent mice at the end of pregnancy, when the serum level of total corticosterone declined (van Zon et al., 1983). This finding has been explained by a possibly reduced CBG production in the liver as a consequence of severe liver pathology at the end of the first week of infection (Eling et al., 1977; van Zon et al., 1978; van Zon et al., 1983).
Though the serum concentration of unbound corticosterone does not increase in pregnant mice that do not recrudesce, as contrasted with human pregnancy, the free corticosterone concentration of mice experiencing a recrudescence at a later point during pregnancy had already increased compared to those that maintained immunity throughout (van Zon et al., 1983).

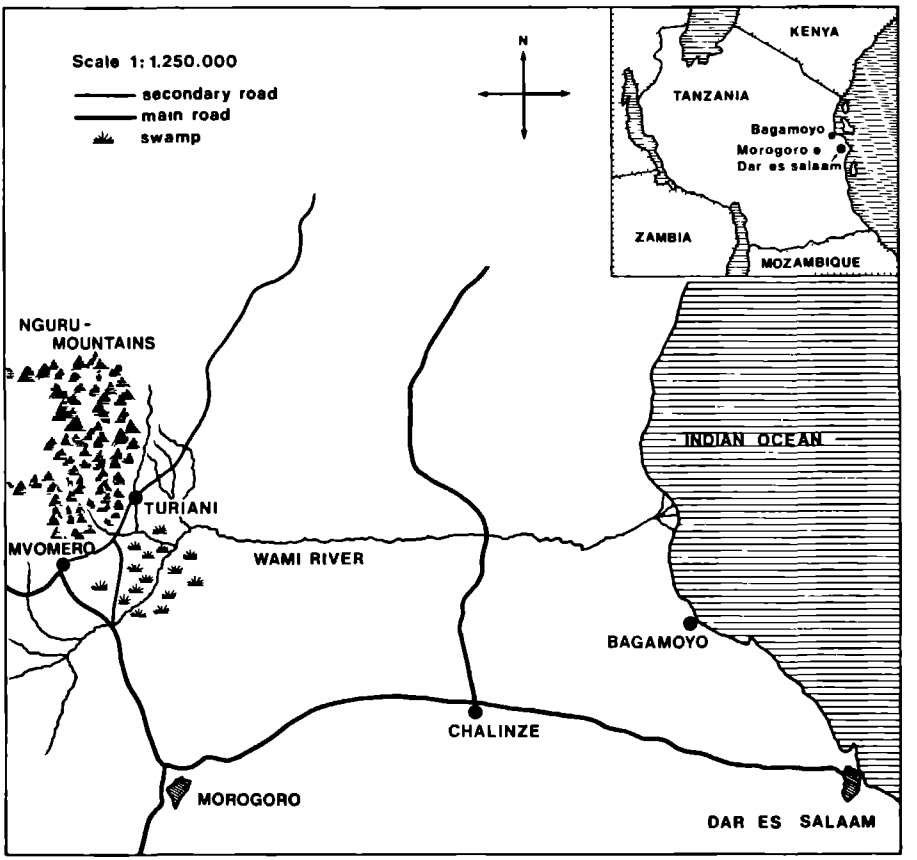
In summary, it appears that the suppression of malaria immunity in pregnant mice is accompanied by an increase of total serum corticosterone as well as of free hormone. The described experiments suggest that corticosterone may act as an immunosuppressive serum factor for malarial immunity during pregnancy.

CHAPTER II

MATERIAL AND METHODS

A: AREA OF STUDY

The material for the study was collected at the Turiani Hospital, a mission hospital nearby the little town of Turiani, Tanzania. The hospital is situated in the densily populated Turiani division, the northern part of the Morogoro District, 300 km inland west from Dar Es Salaam and 100 km north of Morogoro town (see fig.II-1).




legend Fig. II-1; Map of study area

Although the Mzigua and the Mnguu tribes originally belong to this area, a mixture of Bantu tribes, some Massai, and Indians are found here as well nowadays.

During the years of this study the temperature range varied from 35.1°C in february 1982 to 17.7°C in july 1981. The annual rainfall amounts normally to 1200-1600 mm per annum, but in the years of this study it was only 800 mm in 1981 and 1300 mm in 1982 as was measured at Mtibwa Sugar Estate only 2 miles away (Ann.rep.M.S.E. 1980, 1981, 1982). There are two rainy seasons: the long rainy season from March to May and the short rainy season from November till January.

The altitude of the study area at the foot of the Nguru mountains is 300 meters above sea-level. The permanent rivers issuing from these mountains support not only the human but also the mosquito population. At the junction of the foothills and the plain the rivers form swamps, bassins, overgrown streams, and small brooklets with almost standing water. The main malaria vectors Anopheles gambiae and Anopheles funestus cause a continuous malaria transmission throughout the year with some increase shortly after the rainy seasons (Clyde, 1967).

The last malaria survey done by the Tanzanian Malaria Service dates from 1961 and showed that the Turiani area was hyperendemic (Clyde,1967). The predominant malaria species at that time was Plasmodium falciparum (96.4%) while Plasmodium malariae (5.2%) and Plasmodium vivax/ovale (2.8%) occurred at low incidence. In the course of the study the impression was obtained that malaria morbidity in the study area was increasing.

Analogous to Clyde's observations on the annual increase of the proportion of malaria cases relative to all new cases in all Health Institutions in Tanzania (Clyde, 1967) we observed a steady increase in the proportion of malaria cases in the Turiani Hospital over the period 1979-1982 (Table,II-1).

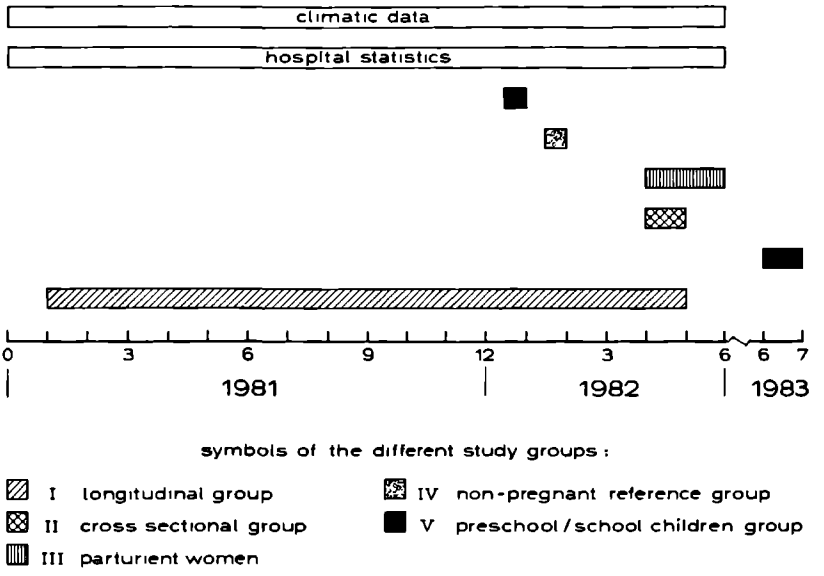
Table II-1
Percentage of malaria cases relative to all new cases in the Out Patient Department of the Turiani Hospital

| | malaria cases/new cases |
|---|---|
| year 1979; | 2632/20.080 = 13.1% |
| year 1980; | 4251/28.342 = 14.9% |
| year 1981; | 5284/27.712 = 19.1% |
| year 1982; | 6696/28.417 = 23.6% |

The observed resistance of the malaria parasite to drugs such as chloroquine and sulfadoxine-pyrimethamine in the Turiani area (Vleugels et al., 1982) also indicates that the situation with respect to malaria in this area is deteriorating.
Malaria was considered to be hyperendemic in the area at the time of the present study (chapter III) and chloroquine was routinely used as the primary prophylactic and therapeutic drug.

B: FORMATION OF STUDY GROUPS

In the Turiani area the pregnant women visit the antenatal clinics of the hospital at irregular intervals depending on e.g. the distance from their home to the hospital, weather conditions, diseases, or amenorrhea. The majority of the pregnant women visit the clinic for the first time at the beginning of the second trimester when they experience the first fetal movements and their family members are becoming aware of their pregnant state by the growing abdomen. The majority will stop these visits when the heavy load hampers them to walk the long distance from home to the clinics and subsequently they will deliver at home. As a consequence of this custom the majority of the women described in the cross-sectional and the longitudinal studies were checked between 20-36 weeks of amenorrhea and are not present in the study group of the parturient women.




legend Fig. II-2; Scheme of the different study groups during the study period

The collection of data in the different study groups during the study period is schematically represented in fig.II-2.

Longitudinal study group (I): During a period of 11 months all pregnant women visiting the antenatal clinics were taken into a longitudinal study. Only pregnant women who suffered from malaria were added to this group and followed up during the next period of five months.

A small proportion of these women delivered at the hospital and only information about the outcome of their pregnancies could be obtained.

Cross-sectional study group (II): At the end of the longitudinal study a cross-sectional study was performed. Within a period of 14 days all pregnant women who visited the antenatal clinic were included in this group. An important difference between the two study groups was the parasitological analysis. In the longitudinal study group a bloodsmear was only made when the mother had clinical symptoms suspicious of malaria, while in the cross-sectional group it was made in all women studied.

Parturient women (III): All parturient women who visited the hospital for delivery during a period of one month formed the last study group of pregnant females.

Non-pregnant reference group (IV): To obtain reference data a group of non-pregnant women was formed and analyzed out of the female hospital employees.

Pre-school and school children (V): To obtain further insight in the local malaria situation at the period of the study a simple survey among pre-school and school children was carried out. Malariometric data from hospital statistics and climatic data, recorded on the Mtibwa Sugar Estate during the study period, were analyzed too.

C: SUBJECTS OF STUDY

1. Longitudinal study group of pregnant women: group I,n=789

All pregnant women who visited the antenatal clinic for the first time during the period 1.2.1981 till 31.12.1981 were registered for this group. To increase the intake of malarious women all those women who started visiting the clinic regulary before 1.2.1981 who suffered from a malaria attack after that date were also registered during this period of 11 months. For the same reason only women who suffered from malaria and who were not yet included in the study were added subsequently for a further period of 5 months (1.1.1982-31.5.1982).

At the first and one of the subsequent visits - usually 4 to 10 weeks later - blood for cortisol measurement was sampled and the following items recorded: age, parity, amenorrhea, fundal height, temperature, spleen size and haemoglobin (Table II-2). Only women with symptoms suspicious for clinical malaria - headache, backpain, pain in the loin and limbs and/or fever, and/or an enlarged spleen - were checked for the presence of parasites by a thick bloodsmear. If parasites were present, the patient was included in the group of women with clinical malaria and otherwise she was included in the group of "non-malarious" women, i.e. women without clinical malaria.

Table II-2
Data collected in the different study groups

| | I | II | III | III(child) | IV | V |
|---|---|---|---|---|---|---|
| spleen size | 0 | 0 | 0 | | 0 | 0 |
| body weight | 0 | | 0 | 0 | | 0 |
| temperature | 0 | 0 | 0 | | | |
| age | 0 | | 0 | 0" | 0 | 0 |
| parity | 0 | 0 | 0 | | 0 | |
| fundal height | 0 | 0 | 0 | | | |
| thick bloodsmear | 0* | 0 | | | 0 | 0 |
| thin bloodsmear | 0* | | 0 | 0 | | 0 |
| haemoglobin | 0 | | 0 | | | |
| cortisol | 0 | 0 | 0 | 0 | 0 | |
| placental tissue | | | 0 | | | |

*these data were only collected in the malarious women
"gestational age according to the Lubchenco (1970) procedure

Table II-3
The number of bloodsamples collected for cortisol analysis at the different visits of study group I

| | |
|---|---|
| first no-malaria visit | 627 |
| second no-malaria visit | 308 |
| first malaria visit | 188 |
| second malaria visit | 12 |
| third malaria visit | 1 |
| total number of samples | 1136 |

Although fever is a symptom inherent to a primary clinical malaria attack, it should be noted that the pattern of fever during a recrudescence can be erratic (Bruce-Chwatt 1963), e.g. remittent fever sometimes imitating tertian periodicity of fever like that of P. vivax may be observed (Wilcocks and

Bahr 1978). Thus, women with clinical malaria examined once during their visit to the antenatal clinic may or may not have a normal body temperature. When the diagnosis was confirmed an additional thin blood smear was made, blood was collected for cortisol analysis, and the above mentioned data recorded. By this approach many women could be sampled for cortisol twice during their pregnancy, and some with malaria attacks were sampled three, four, or even five times. In Table II-3 the number of bloodsamples for cortisol analysis collected at consecutive visits is given. Repeated sampling in groups of malarious and non-malarious pregnant women is given in Table II-4. Malarious women are pregnant

Table II-4
Repeated sampling of non-malarious and malarious pregnant women of study group I

| | |
|---|---|
| non-malarious women; one visit | 340 |
| non-malarious women; two visits | 261 |
| malarious women; one visit | 123 |
| malarious women; two or more visits | 65 |
| total number of patients | 789 |

women with a recorded clinical malaria at one or more of the visits to the ante-natal clinic. Moreover, malarious women can have non-malaria visits as well. Most of these women delivered at home; only 194 came to the hospital in labour. The condition of their newborns was determined by a midwife or a medical officer recording an Apgar score at 1 and 5 minutes after birth (tabel II-5). The body weight and sex of the child were recorded, and the haemoglobin of the mother was measured.

Table II-5
Apgar scoring chart

| Sign | Score 0 | Score 1 | Score 2 |
|---|---|---|---|
| Heart rate | absent | below 100 | over 100 |
| Respiratory effort | absent | weak cry | strong cry |
| Muscle tone | limp | some flexion of extremities | well flexed extremities |
| Reflex response | no response | grimace | cough, sneeze, cry |
| Colour | blue pale-wite | completely blue | body pink, extremities blue |

2. Cross-sectional study group of pregnant women: group II, n=242

All pregnant women who visited the antenatal clinic during a period of two weeks were taken into this cross-sectional study. The following data were recorded: parity, amenorrhea, fundal height, temperature, and spleen size. A thick blood smear was made, and blood was sampled (Table II-2).

3. Parturient women and their offcome: group III, n=52

All women coming to the hospital for delivery during a period of six weeks formed the subjects of this last group. This group was formed irrespective of whether they had visited the antenatal clinic before or whether they had been part of one of the other study groups as well.
All types of deliveries were included, except the caesarian sections. A bloodsample was collected from the mother by venous puncture, and a thin bloodsmear made by fingerprick within a half hour after delivery. The following data were added: amenorrhea, fundal height, parity, haemoglobin value as measured at the last antenatal clinic visit, time and kind of delivery.

The mothers were kept at least three days in hospital. During this period mother and child were observed, and two bloodsamples or more collected from the mother by venous puncture. The time of the samplings was recorded as hours after delivery.

The condition of the newborn was determined according to the Apgar score at 1 and 5 minutes after the delivery; additionally body weight and sex were recorded. The gestational age was assessed using the characteristics suggested by Lubchenco (1970), worked out by the method of Dubowitz (1970), and adapted to the dark skin of the African newborn by Boersma (1979).

From the newborn a bloodsample was collected, and a thin bloodsmear was made by punction of the umbilical vein.

A central cotyledon on the maternal side of the placenta was swept clean with dehydrogenated alcohol before a thin bloodsmear was made from the blood set free after incision of this lobe. From the same cotyledon a biopsy containing the whole placental layer was taken for histopathological examination.

4. Reference group of non-pregnant women: group IV, n=84

Female employees of the hospital volunteered for the study. They formed a non-pregnant control group. Their spleen size was determined, and age and parity recorded. All were of fertile age, non-pregnant, healthy, and did not report recent clinical malaria. Those using any drug during the study period, especially contraceptive drugs (effecting serum cortisol levels; Carr et.al, 1979), were excluded from the study. Moreover thick bloodsmears were made and a blood sample collected for cortisol measurement (Table II-2).

5. Pre-school and primary school children: group V, n=654

To ascertain malaria endemicity children who visited the Mother and Child Health Clinics at the hospital and the children of the primary schools of the Manyinga and Kilimanjaro villages were examined for age, sex, and spleen size.

On another occasion a group of children was seen at the Mother and Child Health Clinics and the Manyinga primary school for examination of spleen size, age, sex, and parasitaemia (Table II-2). The presence of parasites in their blood was checked by thick and thin bloodsmears, and the Plasmodium species was determined.

## D: METHODS USED

### 1. Considerations on terminology

Malaria immunity is considered to comprise all immune responses that protect against proliferation of the parasite to unacceptable levels, and against pathological reactions associated with presence and proliferation of the parasite. Loss of immunity, therefore, may relate to loss of one or more immune responses resulting in one or more disease symptoms, and is not used in an absolute sense.
Malaria immunity is of the premunition type implying an immunological equilibrium between parasite and host. There is an equilibrium on parasitological terms i.e. the number of parasites in the host is adequately restricted, and on clinical terms i.e. pathological effects associated with the presence/proliferation of the parasites. Loss of immunity is a more or less severe or complete disturbance of this equilibrium. Consequently patients with a parasitaemia may or may not exhibit or complain of clinical malaria symptoms. When the patient presented with symptoms suspicious of malaria and exhibited a parasitaemia, she was considered to have clinical malaria in this study.

### 2. Palpation of the spleen

All study subjects - women and children - were physically examined for enlargement of the spleen in the supine position. The enlargement determined according to the classification of Hacket (1944; see Table II-6) was obtained in all non-pregnant subjects, including the children (group V and group IV). In all the pregnant women (study groups I,II,

Table II-6
Classification of spleen size on palpation according to Hackett (WHO Rep. drafting comm. 1963)

| Class number: | Description: |
|---|---|
| 0 | normal spleen; not palpable even on deep inspiration. |
| 1 | spleen palpable only on deep inspiration or at least more than normal inspiration. |
| 2 | spleen palpable on normal breathing but not projected below a horizontal line halfway between the costal margin and the umbilicus. |
| 3 | spleen with lowest palpable point projected more than halfway to the umbilicus but not below a line drawn horizontally through it. |
| 4 | spleen with lowest palpable point below the umbilical level but not projected more than halfway towards a horizontal line through the symphysis pubis. |
| 5 | spleen with the lowest palpable point below the lower limit of class 4. |

III) it was only recorded whether the spleen was palpable or not, since we did not presume that a classification of the enlargement was reliable due to the changed anatomical positions in the abdomen during pregnancy. The spleen rate is the percentage of examined persons with palpable spleens.

3. Assessment of amenorrheal period

Since the mother does not know exactly by day but only by month the last menstrual period, it is very unreliable to use the "anamnestic amenorrhea" to determinate the gestatio-

nal age in the tropics. This "anamnestic amenorrhea" given by the mother had to be combined with the obstetrical estimate: i.e. events such as the experience of the first fetal movements and the registration of fetal heart sounds, physical examination of the mother, and the growth of the fetus. By combining these anamnestic and clinical estimations, we tried to assess the amenorrhea.Being aware of the possible difference of two weeks at each palpation, the amenorrheal period was recorded in an even number of weeks.

4. Bloodsmear examination

All smears were air-dried and stained with May Grünwald-Giemsa's solution. The smears were examined by Miss Lieke Felten, laboratory technician at the Turiani Hospital as well as by the author. The parasites were counted against 200 leucocytes; this number of parasites was multiplied by a factor of 30, assuming a standard leucocyte count of 6000 per $mm^3$. The assumption of a standard leucocyte count of 6000 per $mm^3$ was a general practice in the hospital, obtained from a survey of the local population.
The parasite densities were classified according to the system of Bruce-Chwatt (1958; see Table II-7). The parasite

Table II-7
Classification of parasite densities (Bruce-Chwatt 1958)

| | Parasite count per $mm^3$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | less than 100 | 101-200 | 201-400 | 401-800 | 801-1600 | 1601-3200 | 3201-6400 | 6401-12800 | 12801-25600 | over 25601 |
| Class | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

rate is the percentage of smears showing parasites. The Positive Parasite Density Index (PPDI; WHO 1963) indicates the average parasite density in a number of positive smears.

The PPDI can be calculated by multiplying the frequencies determined for each class by the class number, adding these products and dividing the total by the number of positive slides.

5. Measurement of the body temperature

The temperature of the study subjects was measured by the axillar method. A temperature of 37.0°C. or below was considered normal, while an axillar temperature above 37.0°C. was taken to indicate fever.

6. Measurement of haemoglobin

Haemoglobin levels were measured by the cyanmethaemoglobin method, using a haemoglobin colourimeter (EEL-meter; Dr. Lange photometer LP3) and the values were recorded in grams of haemoglobin per 100 ml (g %).
Normal values of haemoglobin in non-pregnant women range from 11.5 to 16.5 g %, and a pregnant woman was considered to be anaemic if the haemoglobin level was lower than 11.0 g % (6.8mmol/L; WHO 1972).

7. Measurement of total serum cortisol

Because of diurnal changes in serum cortisol levels, all bloodsamples were collected by venous puncture between 11am and 1pm. The samples were stored in a refrigerator for about two hours and then centrifuged for 10 minutes at 10,000 rpm and the serum collected. Subsequently the serum was frozen and stored at -20°C till transportation to the Netherlands. During transport in a pre-cooled Dewar container they were additionally cooled with ice-blocks included in the container during 24 hours. On arrival in Nijmegen they were still frozen and kept at -20°C until analysis.
Measurements were performed in the laboratory of Chemical and Experimental Endocrinology (head Prof. TH. J. Benraad) of the St. Radboud Academic Hospital Nijmegen, the Netherlands.
Total serum cortisol concentrations was determined by two different radio immuno-assays (RIA). All serum samples were assayed in duplicate.

**Cortisol RIA with the CENTRIA system :**

Cortisol concentrations in sera of non-pregnant women were measured by the direct double antibody $^{125}$I-radio immuno-assay in an automated system (CENTRIA System, Union Carbide Clinical Diagnostics, Rye, NY 10580). The rabbit antibody used was raised against cortisol-21-hemisuccinate-BSA, and the second antibody was raised in sheep against rabbit gamma-globulin (Meriadec et al., 1979).

The intra-assay coefficient of variation is given in Table II-8 showing a good variance over the whole range of measured values.

Table II-8

Intra-assay coefficient of variation for five ranges of cortisol values, measured with the CENTRIA-RIA method

| Range;(µmol/L) | Coeff.variarion; |
|---|---|
| 0.07-0.28 | 5.5% |
| 0.28-0.55 | 3.7% |
| 0.55-1.11 | 4.3% |
| 1.11-1.66 | 5.0% |
| 1.66-2.22 | 6.7% |

Table II-9

Values of control sera (µmol/L) measured with the CENTRIA-RIA method in 3 series compared to the values obtained with the same method previously: L=low range sera, M=medium range sera, H=high range sera

| **Sera;** | **previous data;** | | **values of the control sera** | | |
|---|---|---|---|---|---|
| | X | SD | assay 1; | assay 2; | assay 3; |
| L. | 0.14 | 0.02 | 0.14 | 0.16 | 0.14 |
| M. | 0.37 | 0.04 | 0.37 | 0.36 | 0.35 |
| H. | 0.78 | 0.06 | 0.79 | 0.76 | 0.79 |

An inter-assay coefficient of variation is not given, because only 3 series of measurements were done by this method. The results of the control sera, which were included in these assays have been compared to the known distribution obtained earlier with this method (Table II-9). The Table shows a good correlation between the results obtained in the 3 series and the previous data of the same control sera.

**Cortisol RIA with the AUTOPAK system :**

All samples of pregnant women were assayed for total plasma cortisol with the AUTOPAK test system (AUTOPAK cortisol test kit; MICRO-MEDIC Systems). This test is a quantitative radio-immuno-assay for the determination of cortisol in human serum, using cortisol-$I^{125}$-histamin as tracer. Antibodies against cortisol raised in rabbits were affixed to the assay tubes. These tests were performed in the fully automatic CONCEPT-4 (MICRO-MEDIC Systems).

The inter-assay coefficient of variation was determined with five control sera included in each series of measurement (Table II-10). The overall interassay coefficient of variation was 5.1%. As the assayed samples covered a broad range of values the intra-assay coefficient of variation was calculted for five consecutive ranges. The average coefficient was 4.5% (Table II-11).

Table II-10

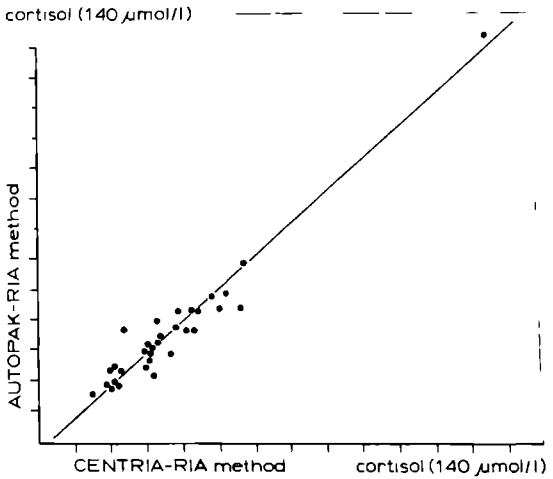
Inter-assay coefficient of variation for the cortisol assays performed with the AUTOPAK method. The 5 control sera (µmol/L) are coded with Roman figures

| Sera; | n; | X | SD | coeff.variation. |
|---|---|---|---|---|
| I | 15 | 0.18 | 0.01 | 6.8% |
| II | 14 | 0.28 | 0.01 | 3.8% |
| III | 15 | 0.37 | 0.02 | 5.8% |
| IV | 15 | 0.76 | 0.04 | 5.5% |
| V | 14 | 0.91 | 0.03 | 3.7% |

Table II-11
Intra-assay coefficient of variation for 5 ranges of cortisol values measured with the AUTOPAK method

| Range;(µmol/L) | coeff.variation. |
|---|---|
| 0.07-0.28 | 3.4% |
| 0.28-0.55 | 4.8% |
| 0.55-1.11 | 4.5% |
| 1.11-1.66 | 4.7% |
| 1.66-2.22 | 5.3% |

**Comparison of the AUTOPAK-method with the CENTRIA-method :**
Because two different RIA-methods were used, 32 sera of non-pregnant women (group IV), which had been assayed with the CENTRIA-method were also assayed with the AUTOPAK-method. The Spearman Rangorder Test was used to calculate the coefficient of correlation, which was 0.97 with a p-value < 0.001 (fig. II-3).




legend Fig. II 3; Correlation between the CENTRIA method and the AUTOPAK method

8. Measurement of unbound cortisol fraction in serum

The fraction of free cortisol in serum was measured in sera of non-pregnant women of the reference group and pregnant women of the longitudinal study group and the study group of parturient women by equilibrium dialysis using radiolabelled cortisol.

Dialysis was carried out in a "Dianorm (Diachema A.G. Rüschlikon, Switzerland) equilibrium dialyser". In this system a semipermeable membrane divides a teflon dialysis cell into two identical compartments. Serum is pipetted into one half-cell and buffer into the other. Membranes were cut from Visking dialysis tubing (assumed molecular weight cut-off 20,000 (Ross 1980), soaked and washed with distilled water and then rinsed in dialysis buffer (phosphate 0.15M pH 7.4). Determination was performed in microcells with a working volume of 2x200 µL and a membrane area of 2 $cm^2$. The $^3H$-cortisol was purified by paper chromatography in the Bush $B_5$-system, eluted in phosphate buffer and used for dialysis. A serum sample of 150 µL was dialyzed against 150 µl phosphate buffer, pH 7.4 containing approximately 200,000 dpm of purified $^3H$-cortisol, for 3 hours at 37°C under continuous rotation.

Following dialysis, 100 µL of the dialysate was pipetted into counting vials for counting of radioactivity. The free fraction was calculated by the following formula:

$$\text{free fraction (\%)} = \frac{C_2}{C_0 - C_2} \times 100$$

where; $C_2$ = radioactivity in dialysate (cpm/100µL).
$C_0$ = radioactivity in phosphate buffer (cpm/100µL).

The inter-assay coefficient of variation was determined by including a control serum in duplicate in each series of measurements. The overall inter-assay coefficient of variation was 6.8%. The intra-assay coefficient of variation was 8.5%.

Effects of procedures and standard conditions (i.e. temperature, dilution of dialysate and tracer contamination) on this equilibrium dialysis have been described previously by dr. A. Ross in his thesis (1980), but were determined and evaluated again in cooperation with dr. A. Ross before assaying these sera. Since no differences were found compared to his previous findings, the same procedure was followed. Moreover, the influence of repeated freezing and thawing, as well the absorption by the different materials of the vials used were measured and appeared to be absent.
Yet the values of control sera and sera of patients in the first two assays were higher than all other series for reasons unknown. Because these two series contained sera of non-pregnant women with and without malaria and pregnant women without malaria, results will only be used for comparison between malarious and non-malarious, non-pregnant women (chapter IV-D) and for comparison between non-malarious pregnant and non-pregnant women (chapter VI-G). No comparison can be made between these results and the results concerning the free fraction of cortisol of pregnant women, obtained from all other assays (chapter VI-G 2,3 and 4; chapter VII-G).
Analysis of the fraction of free cortisol in serum of pregnant women is described in chapter VI-G 2,3, and 4, and comprised 131 samples, obtained from 31 malarious and 32 non-malarious women at one or more occasions.

9. Histopathological analysis of placental tissue

Placental tissue was obtained from all placentae from the subjects in study group III. Before cutting the maternal surface of a central cotyledon the area was wiped clean with dehydrogenated methyl-alcohol. The tissue was stored in 7% neutral formaldehyde and transported to the Netherlands. At the department of Histology and Cytology (head Prof. C. Jerusalem) of the Catholic University of Nijmegen all tissue samples were embedded in paraffin. Sections (5-7µm) were cut and stained with ferri-haematoxylin (Weigert) azofloxin, with Masson's trichrome stain modified by Jerusalem (1963), PAS-haematoxylin, and methylgreen-pyronin.

## E: PROPHYLACTIC ADMINISTRATION OF ANTI-MALARIA AND ANTI-ANAEMIC DRUGS

The following prophylactics were issued to all pregnant women who visited the ante-natal clinics:

a) 300mg chloroquine base per week in Tablets of 150mg each.

b) ferrous sulphate in a dosage of 400-500mg per week.

c) folic acid 10mg per week.

These drugs were given at each visit in a regimen which covered the period between two consecutive visits. Although it is the only method of administration available, it is not believed to provide reliable uninterrupted prophylaxis (Kortmann 1972).

Regular prophylaxis sustained for about two decades has influenced the humoral immunological response measured by the immuno-fluorescent antibody technique (IFA: Onori 1983), but this decrease in IFA levels is not believed to be related to a decrease in the protective humoral immunity (Voller and Wilson 1964; Onori 1982). Prophylactic administration of chloroquine for the duration of the pregnancy lowers the level of malarial immuno-fluorescent antibodies (IFA) (Kortmann 1972), but the level of IgG antibodies which are known to contain protective anti-malarial antibodies (Cohen 1961) is hardly altered during prophylaxis in pregnancy (Gilles 1969; Kortmann 1972).

CHAPTER III

# MALARIA IN THE STUDY AREA

## A: MALARIA ENDEMICITY IN THE STUDY AREA

### 1. Spleen rate

Data on the endemicity of malaria in the Turiani area may be of relevance to the study of malaria during pregnancy.

The grade of endemicity in the study area can be characterized partly by spleen rates of pre-school and primary school children (see material and methods; chapter II-C). A first survey to determine these spleen rates was performed in January 1982, just before the short rainy season (fig. II-2; Table III-1). Spleen rates appeared to be lower at this survey than was expected according to the findings of Clyde (1967). These lower spleen rates may be due to the extreme drought during 1981 (see also chapter II-A; chapter III-B).

A second malaria survey, performed in June 1983 after the long rainy season, revealed higher spleen rates. Results are given in table III-1 and show an overall spleen rate of 41.8% in January 1982, and 49% in June 1983.

Table III-1
Spleen rates of children in the Turiani area

| survey | total group | 0 ≥ 12 months | 1 ≥ 9 years | 9 ≥ 14 years |
|---|---|---|---|---|
| 1982 | 148/354=41.8% | 15/45=33% | 69/159=43.4% | 64/150=42.6% |
| 1983 | 147/300=49% | 44/69=63.8% | 64/106=60.4% | 39/125=31.1% |

The proportion of enlarged spleens in the age group 2 to 9 years was used to designate the degree of endemicity, using WHO cirteria (WHO Rep. drafting committee 1963). According to these criteria the observed spleen rates point to a

mesoendemic area in 1982 (43.4%) and to a hyperendemic malaria area in 1983 (60.4%).

2. Parasite rate

Spleen rate is not the most reliable parameter for malaria prevalence (WHO 1963), since the proportion of palpable spleens in older children and adults can be both low and high in highly endemic areas for reasons not fully understood. Moreover, other tropical diseases can cause enlargement of the spleen, and finally unreliable results are to be expected in the anxious and/or crying child or in the African adult with well developed abdominal muscles. For these reasons parasitaemias were also determined in the survey of June 1983.

Parasite density varied between 60-180.000 parasites per $mm^3$. Parasite rates and PPDI (parasite positive density index) are given in Table III-2. According to a classification of endemicity of malaria based on the parasite rate (WHO 1963), the Turiani area is a holoendemic malaria area.

Table III-2

Parasite rates and PPDI (parasite positive density index) among children in the Turiani area

| | 0≥12 months | 1≥9 years | 9≥14 years |
|---|---|---|---|
| parasite rate | 230/300=76.7% | 64/69=92.8% | 90/106=84.9% | 76/125=60.8% |
| PPDI | 4.0 ± 2.8 | 5.6±2.9 | 4.6±2.5 | 2.0±1.6 |

The following species distribution was found: P. falciparum 100%, P. malariae 4.5% and P. ovale 4.5%. Thus, in all cases of P. malariae or P. ovale, P. falciparum was present too.

B: SEASONAL VARIATION IN THE STUDY AREA

Malaria transmission in the Turiani area, as in most parts of Tanzania, is perennial but varies to some extent with the

season as the result of seasonal variations in rainfall and temperature (Clyde 1967).
Since all studies described in this thesis, except for the 2nd malaria survey in June 1983, extend over a period of nearly 1.5 year, it is interesting to assess the extent to which seasonal variations in transmission may have influenced the observed parasite rates.
Transmission is determined by the daily number of infective bites per person, which was not studied in our investigations. However, this daily number of infective bites per person depends among others upon the mosquito population in the studied area. This mosquito population in turn is related to rainfall and temperature, both of which show seasonal variation.

Available data which may indicate a seasonal variation in transmission are the parasite rates per month in study group I (longitudinal study group), during the period 1-2-1981 to 31-12-1981 (see materials and methods). Other data that can be used to study seasonal fluctuations are the percentage positive bloodsmears subdivided into adults and children per month in our hospital. The results of these analyses are presented in figure III-1.
Since the size of the mosquito population is related to rainfall and temperature, the average daily temperature for each month is graphically represented, and the months with rainfall of over 100mm are indicated as shaded columns in figure III-1.
A larger proportion of positive bloodsmears was found in children than in adults, as could be expected in an endemic malaria area. The percentage positive bloodsmears in non-pregnant adults (males and females) was higher than the percentage malaria visits in pregnant women, which is to be expected, since parasitaemia does not always mean clinical malaria.
Although transmission is perennial, a seasonal variation in parasite rates was observed. The fluctuations seem to be related to rainfall rather than temperature. In addition,

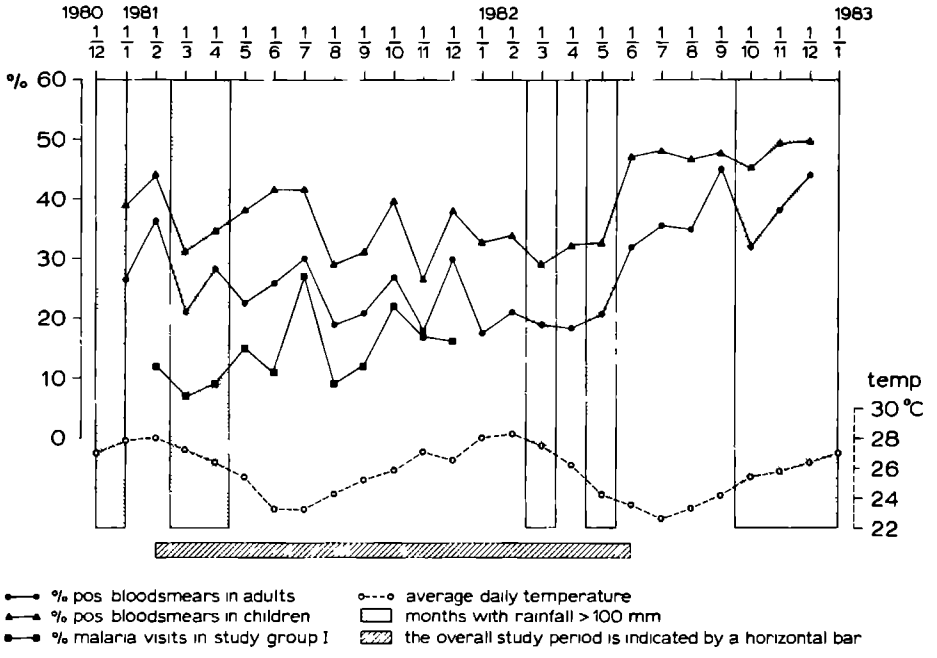



Fig. III-1; Malaria data and seasonal variation of rainfall and temperature during the study period

figure III-1 shows that the parasite rates were lower during the study period February 1981 to June 1982, than during the second half of 1982, which may be related to the unusual drought in 1981, as was already mentioned in chapter II-A. During the first months of 1982 rainfall became normal, and parasite rates during this period rose to levels usually found in hyperendemic malaria areas (chapter II-A).

## C: SUMMARY AND DISCUSSION

The majority of the studies described in this thesis were performed during the unusually dry period of January 1981 to February 1982. The total rainfall was only 721 mm in 1981, but 1200 mm in 1980, and 1340 mm in 1982. The average rainfall is 1400 mm to 1600 mm per annum (see chapter II-A).
A malaria survey at the end of that dry period indicated a meso-endemic malaria area in January 1982, which was not in

accordance with the findings of Clyde (1967) and the statistics of the Turiani hospital. Since Clyde designated Turiani area as a hyperendemic malaria area, the observed differences with the survey in 1982 may be due to this unusually long dry period.

Rainfalls became normal again during the second trimester of 1982, and consequently parasite rates rose to levels known from before 1981. Since immunity of the local population correlates with endemicity, a second malaria survey, performed in June 1983, was needed to determine the endemicity in a year with normal rainfall. The results of this survey indicated that the Turiani area is a holo-endemic malaria area, rather than meso-endemic as found after the unusually dry year 1981.

In spite of the original findings, we may therefore conclude that the integration of the data of various factors, such as malaria prevalence, spleen rate, parasite rate, temperature, and rainfall indicate a "stable malaria" area with high perennial transmission with only slight fluctuations over the years.

All children living in a highly endemic malaria area are likely to be infected by malaria parasites within a few months after birth. Repeated malaria infections during the first years of life provide children with protective immunity. This acquired immunity has to be sustained by the presence of a few malaria parasites, i.e. premunition. Infected mosquito bites are needed during the entire life to protect the older child and adult against clinical malaria attacks.

Thus, a high grade of immunity is only achieved in a "stable malaria" area, and study of the breakdown of malaria immunity during pregnancy is facilitated in such a highly endemic, "stable malaria" area as the Turiani area.

CHAPTER IV

REFERENCE GROUP

## A: INTRODUCTION

A group of female hospital employees and some of their female family members volunteered to take part in this study. They formed study group IV (n=84), a reference group of non-pregnant healthy women for malariometric data such as parasite rate, parasite density, spleen rate, and serum cortisol concentrations.
Since many hospital employees did not originate from the study area, their ethnological background was determined. All were Bantus, but compared to the composition of the local population (2% Mchagga) a larger proportion of them were of the Mchagga (34%).
The age ranged from 16 to 45 years, mean: 25.7 years. Fifty percent of the group were nulliparae and the average parity number was 1.8. None of these women suffered from clinical malaria on the day of examination and sampling. Blood samples were always taken between 11.00 and 12.00 hours and processed as described previously (chapter II).
Parasitaemia was determined in a thick blood smear prepared from a fingerprick.

For some general considerations with regard to the problems of an analysis of the relation between serum cortisol and loss of malaria immunity during pregnancy in a clinical study the reader is referred to the introduction (p. 8).

## B: MALARIOMETRIC DATA

### 1. Parasitaemia

None of the studied women had fever or exhibited any symptom suspicious of malaria. Yet 7 women (8.3%) showed parasites

in their thick blood smear. The parasite density ranged from 400 to 7650 parasites per $mm^3$ (PPDI=5.0±1.5).
The relation between parasitaemia, parasite density, and age (Table IV-1) does not indicate a decline of the parasite rate and density with increasing age, but the figures in the older cohorts are too small to be conclusive, ($p > 0.10$; $X^2$ test).

Table IV-1
Malaria prevalence and parasite density in different age cohorts (study group IV)

| age cohort; | parasite rate; | | parasite density (PPDI); |
|---|---|---|---|
| 16-20 years | 11.5% (3/26) | | 6 |
| 21-25 years | 8.0% (2/25) | 5/66 | 4.5 |
| 26-30 years | 0.0% (0/15) | | - |
| 31-35 years | 14.3% (1/7) | | 5 |
| 36-40 years | 0.0% (0/7) | 2/18 | - |
| 41-45 years | 25.0% (1/4) | | 3 |

2. Spleen rate

In four cases the spleen was of Hackett's size 1, three cases Hackett's size 2 and in one case Hackett's size 3. For the group as a whole a spleen rate of 9.5% was obtained.
The spleen rate in parasite positive women was 1/7=14.3% which was not significantly different from the spleen rate in the parasite negative women, 7/77=9.1% (Fisher exact test; p=0.52).

C: TOTAL SERUM CORTISOL CONCENTRATION

1. Non-pregnant women without parasitaemia

Serum concentration of total cortisol was measured in 77 women without parasitaemia. These cortisol values were plotted

against their age (fig. IV-1) and revealed a slight decrease with increasing age. A linear regression analysis showed that the slope was slightly different from zero (p=0.07). This regression relation was estimated by y=0.46-0.004x, where y=cortisol (µmol/l) and x=age (years) (fig. IV-1).

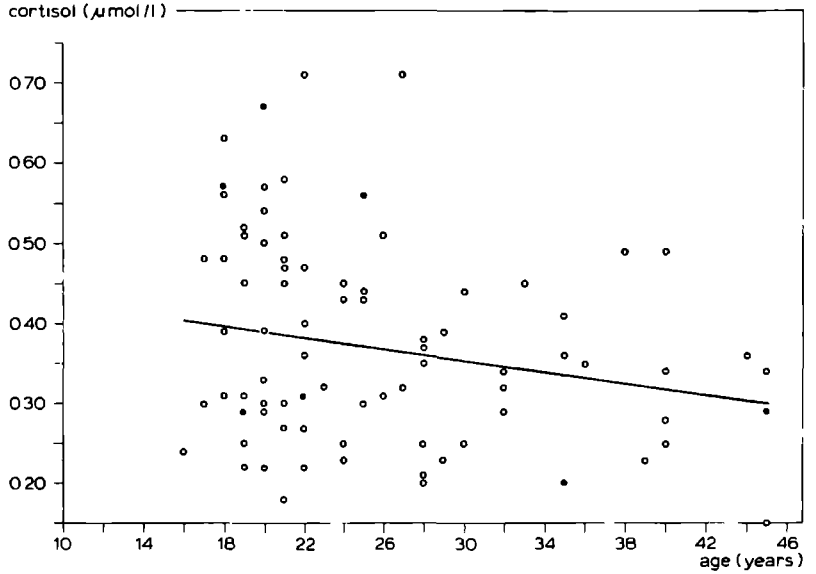



Fig. IV-1;Serum concentration of total cortisol in non-pregnant women in relation to age: o= parasite negative women: • = parasite positive women; values of total cortisol serum concentrations are plotted; only the linear regression line of the cortisol values of parasite negative women is included.

Statistically, there may be an indication, therefore, for a linear relation between cortisol and age.

Considering the broad range of serum concentrations (lowest value 0.15 µ mol/l and the highest value 0.71 µ mol/l) some of these values are suspected to be outliers. However, according to the test procedure of Ellenberg (1976) used for showing one single outlier in a general linear model, the most extreme cortisol values with the largest standardized residual did not differ significantly from the expected cortisol value for the age concerned (p=0.28), nor could other measurements be eliminated as outliers after removing the most extreme value and repeating the procedure.

2. Non-pregnant women with parasitaemia

The total serum cortisol concentrations of parasite positive women were measured, plotted against age, and compared to those of the parasite negative women (fig. IV-1). Again, a linear relation between cortisol and age was obtained for the parasite positive women. The hypothesis of equal slopes for both lines was tested and could not be rejected ($p=0.25$).

The joint test for the hypothesis that both lines have the same intercept and slope yielded a p-value of 0.34.

## D: FREE FRACTION OF SERUM CORTISOL

The free fraction of serum cortisol was measured in all women with parasitaemia (n=7) and compared to values of an age matched group of parasite negative women (n=14) (Table IV-2).

The total serum cortisol concentration of these parasite negative women (n=14) differs neither from the total serum cortisol concentration of all studied parasite negative women (n=77; Student's t-test, 2-tailed $p > 0.10$), nor from the total serum cortisol concentration of the parasite positive women (n=7; Student's t-test, 2-tailed $p > 0.10$).

The parasite positive women had a significantly higher free fraction of serum cortisol than the parasite negative women.

Table IV-2

Free fraction of serum cortisol in non-pregnant women

| | n: | X | SD |
|---|---|---|---|
| parasite positive women: | 7 | 6.1% | 1.1% |
| parasite negative women: | 14 | 4.8% | 0.9% |

(Student's t-test, 2-tailed $p < 0.005$).

The parasite rate (8.3%) in adult non-pregnant women was lower than in preschool and school children (study group V: 76.7%). This finding is characteristic for a highly endemic malaria area and supports the conclusion of chapter III.
As contrasted with data from the literature (Mackay 1935, in Bruce-Chwatt 1963; Wilson 1936), neither parasite rate nor parasite density were significantly correlated with age, though the data are too limited to draw final conclusions.
Spleen enlargement (9.5%) in non-pregnant females was found less frequently than in children of study group V (45.4%), and the spleen rate of parasite positive women did not differ from that of parasite negative women. Together with the observation that none of the parasite positive women suffered from clinical malaria (no fever or other clinical symptoms) this indicates that all possessed sufficient malaria immunity at the moment of study.
Data on total serum cortisol levels of non-pregnant women showed a tendency for linear reduction with age.
In the range of 16 to 45 years covered in this group a reduction of approximately 20% was observed. Similar data are not available from e.g. Caucasian women; on the contrary, Everitt (1980) and Asnis (1981) claim that cortisol levels remain constant in this age group, and Apter et al., (1979) observed an increase in cortisol levels in the post-menarchal period compared to the pre-menarchal period.
These levels are still present at old age (Everitt 1980). The same holds for the circadian changes (Dean and Felton, 1979), though others (Milu et al., 1978) reported the peaks in the circadian rhytm to flatten with age.
Spar and Gerner (1982) on the other hand described a slight decrease of total serum cortisol with age in the elderly (70 to 80 years). They found a correlation coefficient of 0.22 and $0.5 < p < 1.0$.
More data are needed before valid comparisons can be made. A decreasing burden of diseases (not restricted to malaria)

with age, which may be more relevant in African populations, could be one of the possible explanations for the age dependent decrease of cortisol levels.

Such a hypothesis is not supported by the data on total cortisol in parasite positive versus parasite negative women. The finding of significantly higher free cortisol levels in parasite positive women, however, is in line with the hypothesis. Finally it should be recognized that the investigated groups are small, and firm conclusions cannot be drawn.

CHAPTER V

CROSS-SECTIONAL STUDY GROUP

## A: INTRODUCTION

All pregnant women visiting the antenatal clinic during a period of 2 weeks were included in a cross-sectional study group. In this group malaria was analysed in relation to parity, amenorrhea, fever, spleen enlargement, and serum cortisol concentrations. For material and methods see chapter II.
For some general considerations with regard to the problems of an analysis of the relation between serum cortisol and loss of malaria immunity during pregnancy in a clinical study the reader is referred to the introduction (p. 8).

## B: MALARIA PREVALENCE

### 1. Malaria prevalence in relation to parity

The parasite rate in nulliparous and multiparous women is given in Table V-1, and the parasite rate in relation to parity in Table V-2. Results show that nulliparous women have parasites in their blood more frequently than multiparous women (Table V-1) and the parasite rate decreases significantly with increasing parity (Table V-2: van Eeden test; p=0.003).

### 2. Malaria prevalence in relation to amenorrhea

The amenorrhea was determined from the combined results of the "anamnestic amenorrhea" and the fundal height and, if possible, compared with the results obtained at previous visits. The amenorrhea was recorded in an even number of weeks and ranged from 12 to 38 weeks in this study group. Owing to limited numbers of women in some amenorrheal periods the range was subdivided in larger subclasses.

Table V-1
Parasite rate in nulliparae and multiparae of the cross-sectional study group (group II)

| | | PARASITE POS. |
|---|---|---|
| total group: | n=242 (100%) | 39 (16.1%) |
| nulliparae: | n=49 (20.2%) | 14 (28.6%)* |
| multiparae: | n=193 (79.8%) | 25 (12.9%)* |

*($\chi^2$ test; $p < 0.01$)

Table V-2
Relation between parity and parasite rate in the cross-sectional study (group II)

| parity: | 0 | I | II | III | IV | V |
|---|---|---|---|---|---|---|
| total number per parity: | 49 | 41 | 38 | 30 | 21 | 63 |
| parasite pos. women: | 14 | 8 | 5 | 6 | 1 | 5 |
| parasite rate (%): | 29 | 20 | 13 | 20 | 5 | 8 |

Table V-3
Relation of parasite rate and amenorrhea in the cross-sectional study (group II)

| amenorrhea: | total number: | PARASITE POS.: |
|---|---|---|
| 12-18 weeks | 5 | 1 (20%) |
| 20-22 weeks | 28 | 4 (14.3%) |
| 24-26 weeks | 62 | 14 (22.6%) |
| 28-30 weeks | 64 | 8 (12.5%) |
| 32-34 weeks | 54 | 8 (14.8%) |
| 36-38 weeks | 29 | 4 (13.8%) |

The relation between parasite rate and amenorrhea is given in Table V-3. The results suggest that the parasite rate was highest in the periods of 12 to 18 weeks and 24 to 26 weeks, but statistical analysis neither showed differences in probability of malaria between the mentioned periods ($X^2$-test; p=0.25), nor revealed a steady decrease with increasing amenorrhea (van Eeden test; p=0.67).

C: SPLEEN ENLARGEMENT AND FEVER

1. Spleen enlargement

Spleen enlargement in the cross-sectional group in relation to parasitaemia is depicted in Table V-4. An enlarged spleen was palpated in 8 women. The group of women with parasitaemia had a significantly higher spleen rate than the group without parasitaemia.

Table V-4
Parasitaemia and spleen rate in the cross-sectional study

| | | PALPABLE SPLEEN: |
|---|---|---|
| total group: | n=242 | 8 (3.3%) |
| parasite negative women: | n=203 | 4 (2.0%) |
| parasite positive women: | n=39 | 4 (10.3%) |

(Fisher exact test; p=0.03)

Table V-5
Parasitaemia and fever in the cross-sectional study

| | | FEVER: |
|---|---|---|
| total group: | n=242 | 7 (2.9%) |
| parasite negative women: | n=203 | 4 (1.9%) |
| parasite positive women: | n=39 | 3 (7.7%) |

(Fisher exact test; p=0.09)

2. Fever

Seven women had fever, i.e. an axillar temperature above 37.0°C. A significant difference could not be found between women with and without parasites (Table V-5).

## D: TOTAL SERUM CORTISOL

1. Parasitaemia and the serum concentration of total cortisol

Total cortisol concentration of parasite negative women were compared to the total cortisol concentration of parasite positive women and in relation to the gestational period, but independent of parity (fig. V-1).

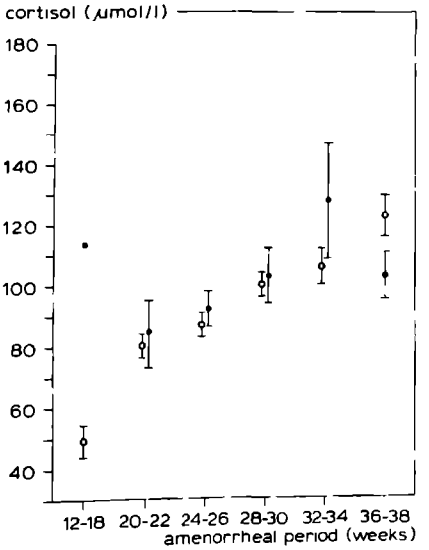



Fig. V-1; Serum concentration of total cortisol in parasite positive and negative women: parasite positive women are represented by solid circles (●), parasite negative women by open circles (○); data represent mean ± SEM. Differences between the mean cortisol concentrations of parasite positive and parasite negative women were analysed by the Student's t-test with variance pooled in each amenorrheal class

Cortisol concentration increases during gestation, and it is obvious that the range of the serum cortisol levels of parasite negative women increases during gestation. Although the

mean cortisol level of the parasite positive women was higher than the corresponding values of the parasite negative women in four amenorrheal periods, this difference is not significant.

Comparison of the serum levels of total cortisol between parasite negative and positive women after subdivision in nulliparae and multiparae revealed no difference among multiparous women (fig. V-2), but higher cortisol values were found among parasite positive nulliparous women, differing significantly in the period of 28 to 30 weeks (fig. V-2).

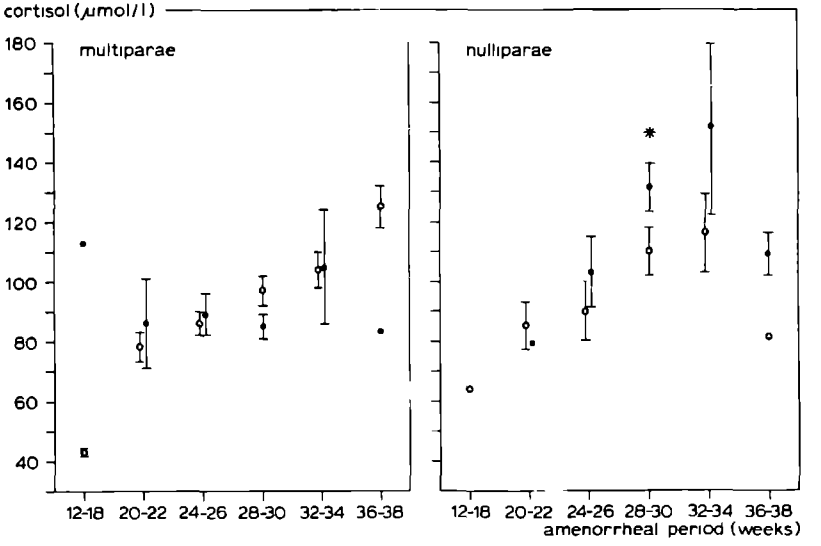



Fig. V-2; Serum concentration of total cortisol in multiparae and nulliparae: parasite positive women are represented by solid circles (●), parasite negative women by open circle (○); data represent mean ± SEM. Differences between the mean cortisol concentrations of parasite positive and parasite negative women were analysed by the Student's t-test with variance pooled in each amenorrheal class and the significance expressed by a 2-tailed p-value: *=$p<0.05$

## 2. Parity and the serum concentration of total cortisol

Though the differences are not significant, total cortisol concentrations tend to be higher in parasite negative nulliparous women than in parasite negative multiparous women (fig. V-3). This difference between nulliparae and multi-

parae was significant at the 5% level in the period 28 to 30 weeks in parasite positive women (fig. V-3).

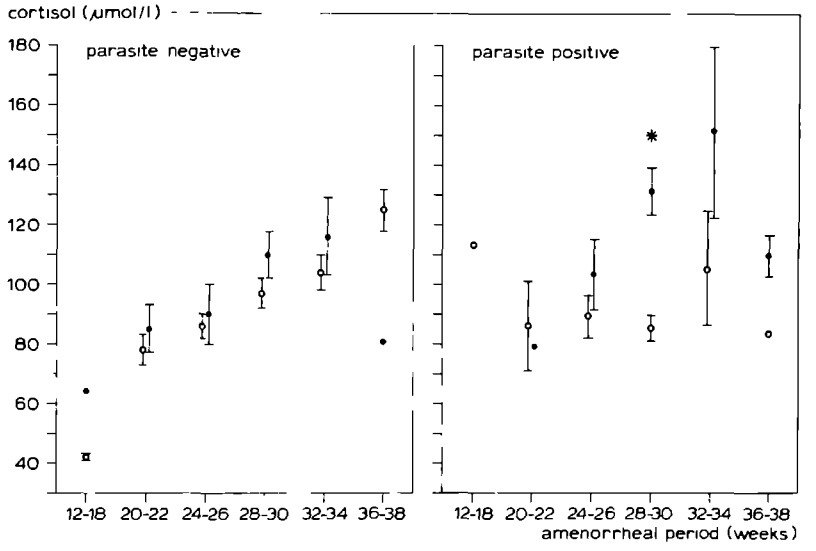



Fig. V-3; Serum concentration of total cortisol in parasite negative and parasite positive women: results of nulliparous women are represented by solid circles (●), those of multiparous women by open circles (○); data are given as mean ± SEM. Differences between the mean cortisol concentrations of nulliparous and multiparous women were analysed by the Student's t-test with variance pooled in each amenorrheal class and significance expressed by a 2-tailed p-value: *=p<0.05

Since we did not consider age in the cross-sectional study, we cannot differentiate between the separate effects of parity and age on the cortisol concentration.

## 3. Parasite density and the serum concentration of total cortisol

*Parasite densities were classified according to Bruce-Chwatt* (see chapter II), and further divided into a group of low (parasite density ≤ 4) and a group with high parasitaemia (parasite density ≥ 5). The distinction between low and high parasite density is arbitrarily made. There is no consensus concerning parasite density used for the limit between low and high grade of parasitaemia in the literature (Bruce-Chwatt 1963).

The mean cortisol concentrations of these groups in relation to the gestational period are compared in Table V-6. A higher parasite density did not correlate with a higher mean cortisol concentration in all amenorrheal periods. Also, if

Table V-6
Parasite density and total cortisol concentrations in the cross-sectional study (group II)

| | **PARASITE DENSITY ≤ 4** | | | | **PARASITE DENSITY ≥ 5** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (total cortisol concentrations) | | | | | | | | |
| amenorrhea:* | n: | x: | S.D. | SEM | n: | x: | S.D. | SEM | p+ |
| 12-18 weeks | 1 | 1.13 | - | - | - | - | - | - | - |
| 20-22 weeks | 3 | 0.92 | 0.21 | 0.12 | 1 | 0.60 | - | - | >0.10 |
| 24-26 weeks | 4 | 1.03 | 0.36 | 0.18 | 10 | 0.88 | 0.10 | 0.03 | >0.10 |
| 28-30 weeks | 4 | 0.91 | 0.23 | 0.12 | 4 | 1.14 | 0.22 | 0.11 | >0.10 |
| 32-34 weeks | 2 | 1.10 | 0.22 | 0.16 | 6 | 1.34 | 0.58 | 0.24 | >0.10 |
| 36-38 weeks | - | - | - | - | 4 | 1.03 | 0.15 | 0.08 | - |

*; Due to the small number of parasite positive women at some amenorrheal weeks, we had to combine them in the described 5 amenorrheal periods.

+; Student's t-test with a variance pooled for each amenorrheal class was used; 2-tailed probability is given.

a different parasite density is chosen for division of the results into a group ≤ 3 and a group ≥ 4, the same results are found (data not shown).

There were more nulliparous women than multiparous women in the period of 28 to 34 weeks. This may contribute to the observed small difference, since the mean PPDI of nulliparae was 5.2±1.6 and that of multiparae 4.8±1.8 (mean PPDI of the total study; 4.9±1.8)

Asymptomatic parasitaemia is common in an indigenous adult population of tropical Africa (Bruce-Chwatt 1963), and may be an expression of the premunition model of malaria immunity in which the presence of parasites is needed to maintain immunity.
In this cross-sectional study we did not account for clinical symptoms of overt clinical malaria - headache, fever, chills, pain in limbs and back combined with parasitaemia. Consequently, the group of parasite positive women comprises a mixture of women with asymptomatic parasitaemia and women who suffered from clinical malaria.
The relevance of fever as a symptom of malaria in immune persons is low (Bruce-Chwatt 1963). Fever was equally present in parasite positive and negative women in this study group. This supports the observation of Bruce-Chwatt (1963) that fever per sé is not related to the presence of parasites.

Palpation of the spleen in an African adult is difficult, due to the well developed abdominal muscles. The changing anatomical positions during pregnancy and the increased muscle tone of the stretched abdominal wall makes palpation of the spleen during pregnancy almost impossible and an unreliable diagnostic symptom. In our opinion this explains the lower spleen rate (3.3%) in the pregnant study group as compared to the non-pregnant study group (group IV) with a spleen rate of 9.5%.
The spleen rate in the parasite positive pregnant women is higher compared to that of the parasite negative pregnant women, but we have to consider these results with caution, since the numbers are very small, as well as the mentioned doubtful reliability of results of spleen palpations: this problem is also dealt with in chapter VII. A possible explanation for the observed significant difference could be that pregnant women suffering from malaria have temporarily a larger spleen which is more easily detectable.

A decreasing parasite rate with increasing parity confirms observations of other studies (chapter I). A remarkable drop in parasite rate after the first pregnancy points to a substantial difference between resistance in nulliparae and multiparae. A decreasing parasite rate in multiparous women may be related to age, since decreasing parasite rates with increasing age have been described before (Bruce-Chwatt 1963) and is also suggested by the results of this study (chapter IV).

Parasite positive women had a higher total cortisol concentration than did parasite negative women. This difference was more marked in nulliparous women.
The observed differences in serum cortisol levels were mostly not significant for each amenorrheal period, which may be due to a lack of differentiation between clinical malaria (i.e. loss of immunity) and asymptomatic parasitaemia, a common phenomenenon in an indigenous adult population.

# CHAPTER VI

# LONGITUDINAL STUDY GROUP

## A: INTRODUCTION

Although we aimed to study pregnant women visiting the antenatal clinic longitudinally, due to local customs a substantial number of women could be examined and sampled only once. Whether analysis was performed on data from repeated visits or data from single visits as well is indicated in the appropriate sections.
For some general considerations with regard to the problems of an analysis of the relation between serum cortisol and loss of malaria immunity during pregnancy in a clinical study the reader is referred to the introduction (p. 8).

Native adults living in a highly endemic malaria area usually have parasites in their blood, but frequently without symptoms of clinical malaria (see also chapters IV, V). A challenge with malaria parasites does not always cause a clinical infection, which indicates that malaria immunity is intact. Thus, on the one hand a positive blood smear apparently does not necessarily indicate a clinical malaria infection, i.e. loss of malaria immunity, whereas on the other hand the presence of fever or other symptoms indicative of malaria, e.g. headache, pain in joints/limbs/back, general malaise, enlarged spleen, do not always point to an actual clinical malaria infection (chapter IV, V).
In this study the diagnosis of clinical malaria, i.e. loss of immunity, was first made on the clinical picture and confirmed by a positive thick bloodsmear, after which a thin bloodsmear was added.
The control group therefore comprises women without actual clinical malaria. This does not exclude the possibility that they actually have an elevated number of parasites (see also chapter II-C).

In the cross-sectional study the PPDI (positive parasite density index) was determined from parasite positive women independent from an analysis of clinical symptoms. Clinical malaria was included as a selection criterium in the longitudinal study to approach the problem of loss of immunity during pregnancy versus premunity. This approach of the patients data resulted in a PPDI of 5.9±2.1 in the longitudinal study (n=159) in comparison to 4.9±1.8 in the cross-sectional group (n=39) (Student's t-test 2 paired $p<0.001$).
Fever did not occur significantly more frequently in the parasite positive women than in the parasite negative women of the cross-sectional group (chapter V), but this difference was highly significant in the longitudinal group (Fisher exact test; $p<0.001$: Table VI-16).

An enlarged spleen was not considered to be a symptom of clinical malaria in non-pregnant women, native to this highly endemic malaria area. According to Bruce-Chwatt (1963) results of spleen palpation are inconsistent in non-pregnant women.
In pregnant women palpation of the spleen is also difficult (Kortmann 1972). In our study groups the PPDI from the longitudinal and the cross-sectional group of pregnant women (5.9 and 4.9 resp.) compared with that of the non-pregnant group (5.0) did not correlate with the spleen rates in these groups (3.3% and 2.4% resp. compared to 9.3%). Here we conclude that the spleen rate can not be used as a valid criterion for the diagnosis of acute malaria in pregnant women. The value of the spleen rate will be discussed further elsewhere (chapter VII).

## B: PREVALENCE OF CLINICAL MALARIA

### 1. Prevalence of clinical malaria in relation to parity and age

In the relation between parity and clinical malaria two factors seem important: the possible effect of parity and age

on changes in immunity. Analysis of the relation between parity and clinical malaria in this longitudinal study group showed that nulliparous women developed clinical malaria significantly more frequently (36%) than multiparous women (19%: Table VI-1).

Table VI-1
Prevalence of clinical malaria and parity

| Parity: | total number: | malaria patients: |
|---|---|---|
| nulliparae: | 188 | 68 (36%) |
| multiparae: | 580 | 113 (19%) |

(Fisher exact test; $p<0.05$)

When nulliparous women were divided into 2 arbitrarily chosen complementary age classes the frequency of clinical malaria in these classes was the same (Table VI-2).

Table VI-2
Prevalence of clinical malaria and age in nulliparous women

| Age: | $n_1/n_2$: | |
|---|---|---|
| 15-24 yrs. | 63/174 | (36%) |
| 25-45 yrs. | 5/14 | (36%) |

$n_1$=number of malaria cases
$n_2$=total number of cases

To analyse the relation between parity and malaria further, the multiparous women were divided into three parity classes ($1 \leq 2$; 3 or 4; $\geq 5$: Table VI-3). The data indicate that prevalence of clinical malaria decreases significantly with increasing parity. When the same parity classes were subdi-

vided into age classes 15 to 24 years, 25 to 34 years, and 35 to 45 years, however, this parity dependent decrease was no longer found (Table VI-3).

Table VI-3
Prevalence of clinical malaria and parity in multiparous women

| parity: | all ages: $n_1/n_2$ | 15≤24yrs: $n_1/n_2$ | 25≤34yrs: $n_1/n_2$ | 35≤45yrs: $n_1/n_2$ |
|---|---|---|---|---|
| 1 ≤ 2; | 51/212 | 40/165 | 11/44 | 0/3 |
| 3 or 4; | 36/185 | 7/31 | 28/140 | 1/14 |
| ≥ 5; | 26/183 | 0/3 | 16/88 | 10/92 |
| | p=0.05 | p=0.61 | p=0.65 | p=0.77 |

(Fisher exact test; $n_1$=number of malaria cases
$n_2$=total number of cases)

Table VI-4
Prevalence of clinical malaria and age in multiparous women

| age: | all women: | PARITY CLASS | | |
|---|---|---|---|---|
| | | 1 ≤ 2: | 3 or 4: | ≥5: |
| | $n_1/n_2$ | $n_1/n_2$ | $n_1/n_2$ | $n_1/n_2$ |
| 15≤24yrs | 47/199 | 40/165 | 7/31 | 0/3 |
| 25≤34yrs | 55/272 | 11/44 | 28/140 | 16/88 |
| 35≤45yrs | 11/109 | 0/3 | 1/14 | 10/92 |
| | p=0.02 | p=0.61 | p=0.46 | p=0.29 |

(Fisher exact test; $n_1$=number of malaria cases
$n_2$=total number of cases)

When multiparous women were subdivided in age classes a significantly decreasing prevalence of clinical malaria was found with increasing age (Table VI-4). When the age classes were subdivided into parity classes (Table VI-4), the signi-

ficance of the age dependent decrease in prevalence of clinical malaria was lost again.
Since parity is known to be related to age, the data on clinical-malaria prevalence, parity and age were also analysed in a "three-way contingency" table. Statistical analysis was done with the "likelihood ratio $X^2$-test (L.R. test) and the "goodness of fit $X^2$-test of Pearson" (Pearson test) in a *loglinear model*.
The hypothesis that clinical malaria and age were conditionally independent (i.e. independent from parity class) could not be rejected (L.R. p=0.37: Pearson p=0.54). The hypothesis that clinical malaria and parity were conditionally independent (i.e. independent for any age class) could not be rejected (L.R. p=0.88: Pearson p=0.77). Parity and age were strongly correlated (L.R. p<0.001: Pearson p<0.001).
Prevalence of clinical malaria is significantly higher in nulliparae relative to multiparae. The higher prevalence in nulliparae is independent of age. The possibility remains however, that in multiparae clinical-malaria prevalence decreases with age and parity.
These results may support the view that significant immunological changes are to be expected in nulliparae relative to multiparae, and that a further reinforcement may be obtained with increasing age and parity.

## 2. Prevalence of clinical malaria in relation to duration of amenorrhea

An increased prevalence of clinical malaria during pregnancy raises the question whether loss of malaria immunity is related to the duration of amenorrhea. The overall picture of visits with or without clinical malaria (fig. VI-1), suggests that the frequency of visits with clinical malaria is related to amenorrhea: clinical-malaria prevalence is higher during the first trimester and at the end of the third trimester. All women visiting the ante-natal clinic were provided with antimalaria drugs for prophylaxis. This prophylactic administration can reduce prevalence of clinical malaria later on during pregnancy. To exclude this effect the rela-

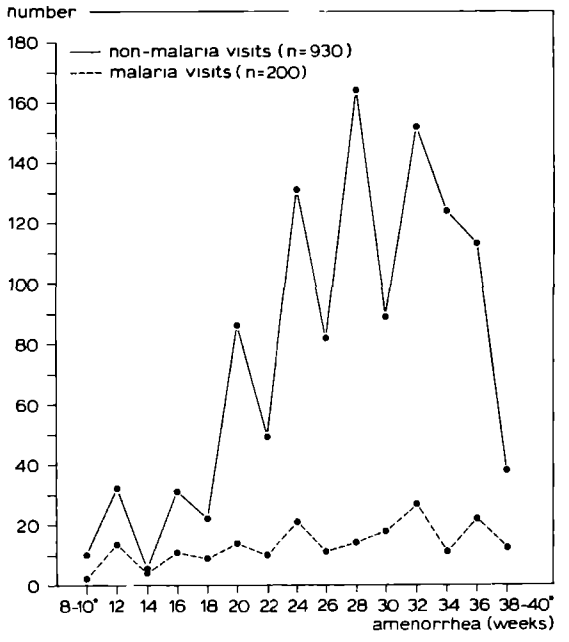



Fig. VI-1; Malaria negative and malaria positive visits in the longitudinal study group in relation to duration of amenorrhea: *due to small numbers, visits between 8 to 10 weeks and 38 to 40 weeks were grouped together.

tion between clinical malaria and amenorrhea was determined from the first visit data of women who did, and who did not reveal clinical malaria during this study. Out of these data the proportion of first malaria visits in relation to amenorrhea was determined for nulliparae and multiparae separately and is presented in figure VI-2.

According to a $X^2$-test for homogenicity the chance of a visit with clinical malaria at each amenorrhea was not the same, neither for the total group ($p<0.001$), nor for nulliparae ($p=0.02$) or multiparae ($p<0.001$) separately.

As mentioned before (chapter II) healthy pregnant women in Turiani area tend to visit the antenatal clinic for the first time at the end of the first trimester or at the beginning of the second trimester. Due to this habit of women without symptoms of clinical malaria the frequency of non-malaria visits increases in the second and the third trimester, thereby decreasing the prevalence of clinical

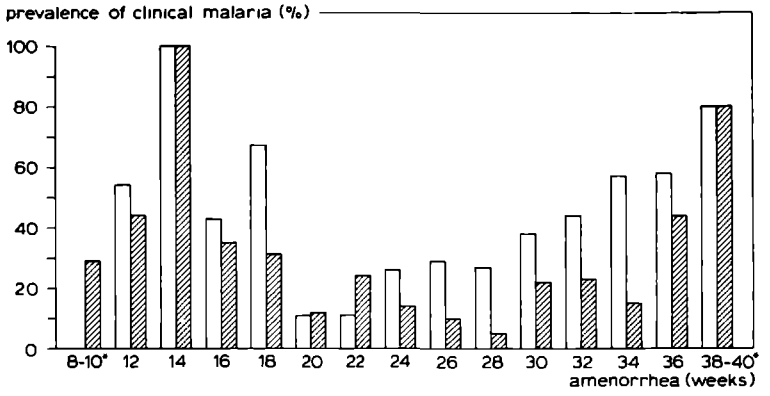



Fig. VI-2: Prevalence of clinical malaria in relation to duration of amenorrhea: □ = malaria prevalence of nulliparae (n=192): ▨ = malaria prevalence of multiparae (n=588): *due to small numbers, visits between 8 to 10 weeks and 38 to 40 weeks were grouped together.

malaria in these periods. To exclude this effect only those women with clinical malaria, who were sampled at least twice were analysed. Their first malaria visit and their first non-malaria visit were taken for the calculations of the percentages of clinical-malaria visits in relation to amenorrhea (Table VI-5). Since women of the malarious group, who were only sampled at their visits with clinical malaria and not at their non-malaria visits as well as women with visits with unknown amenorrhea were excluded, only 60 women were available for this analysis.

Table VI-5
Malaria and non-malaria visits of longitudinally studied women with clinical malaria related to amenorrhea

| amenorrhea: | malaria visits: $n_1/n_2$ | |
|---|---|---|
| ≤16 weeks: | 15/21 | (71%) |
| 18≤26 weeks: | 19/38 | (50%) |
| 28≤40 weeks: | 26/61 | (43%) |

$n_1$=number of malaria visits; $n_2$=number of total visits

The data of this Table (VI-5) add to the significance of the observed differences in prevalence of clinical malaria at different amenorrheal periods described above (see also fig.VI-2).

C; PARASITE DENSITY

1. Parasite density and parity

The positive parasite density index (PPDI) was calculated from the data of nulliparous and multiparous women at their first malaria visit. The PPDI from nulliparae was not significantly different from that of multiparae (Table VI-6). The overall PPDI of all women at their first visit with clinical malaria was 5.9±2.1.

Table VI-6
Positive parasite density index (PPDI) at the first malaria visit and parity

| | n: | PPDI X ± SD |
|---|---|---|
| nulliparous women; | 60 | 6.0±2.0 |
| multiparous women; | 99 | 5.9±2.1 |

(Student's t-test: p=0.76)

2. Parasite density and amenorrhea

The mean PPDI of all first malaria visits was calculated for each amenorrheal period (Table VI-7). The hypothesis that the mean PPDI for the different amenorrheal periods are the same cannot be rejected according to an one-way analysis of variance (p=0.35).

Table VI-7
Positive parasite density index (PPDI) at the first malaria visit and amenorrhea

| amenorrhea (weeks); | n: | PPDI X ± SD |
|---|---|---|
| 8 - 10 | 1 | 3.0 |
| 12 | 13 | 5.8±2.4 |
| 14 | 4 | 7.3±1.9 |
| 16 | 9 | 5.3±2.5 |
| 18 | 7 | 5.4±1.1 |
| 20 | 11 | 6.0±2.1 |
| 22 | 7 | 5.3±2.4 |
| 24 | 18 | 6.4±1.9 |
| 26 | 11 | 7.1±1.8 |
| 28 | 10 | 6.8±1.5 |
| 30 | 14 | 5.1±2.1 |
| 32 | 22 | 5.9±1.9 |
| 34 | 10 | 5.8±1.7 |
| 36 | 12 | 5.5±2.5 |
| 38 - 40 | 10 | 5.5±2.3 |

D; INFLUENCES OF CLINICAL MALARIA ON THE PREGNANT MOTHER AND ON THE NEWBORN

1. Haemoglobin levels during pregnancy

The possible effect of clinical malaria on haemoglobin levels during pregnancy was studied comparing haemoglobin concentrations of pregnant women who obviously did not have clinical malaria during their first visit with haemoglobin concentrations of women who presented with clinical malaria at the time of introduction in this study. Only these haemoglobin values were analysed to avoid an effect of anti-anaemic and prophylactic malaria drugs given routinely in the antenatal clinic.

A haemoglobin concentration of less than 11.0 g % was taken to indicate anaemia in pregnant women (WHO 1972).
Table VI-8 shows that the frequency of anaemia in nulliparae without clinical malaria (94/119=79%) is comparable to that in multiparae without clinical malaria (366/456=80%). Parity as such has no influence on the frequency of anaemia.
Analysis of the frequency of anaemia among nulliparae and multiparae with clinical malaria, indicates a greater probability of anaemia (p=0.09) in nulliparae with clinical malaria (Table VI-8).

Table VI-8
Number and percentage of anaemic patients (Hb < 11.0 g%) in nulliparae and multiparae with or without clinical malaria

| CLINICAL | NULLIPARAE | | | MULTIPARAE | | |
|---|---|---|---|---|---|---|
| MALARIA: | n: | Hb<11.0 | Hb≥11.0 | n: | Hb<11.0 | Hb≥11.0 |
| no: | 119 | 94 (79%) | 25 | 456 | 366 (80%) | 90 |
| yes: | 54 | 49 (91%) | 5 | 89 | 69 (78%) | 20 |
| Fisher exact test: | | p=0.09 | | | p=0.66 | |

Subsequently, the mean Hb level observed in nulliparae and multiparae in relation to clinical malaria was analysed (Table VI-9) by a two-way analysis of variance (complete model). The interaction hypothesis that the difference between the mean Hb concentration of women with or without clinical malaria is the same for nulliparae and multiparae could not be rejected (p=0.98). Thus, the difference in Hb concentration related to clinical malaria is probably independent of parity.
Furthermore, a significantly lower mean Hb concentration was found for pregnant women with clinical malaria compared to those without clinical malaria (p=0.001), and an indication for a lower value in nulliparae compared to multiparae (p=0.07). In view of the absence of interaction in a two-way

Table VI-9
Mean Hb concentrations (g%) related to parity and clinical malaria

| | NULLIPARAE X ± SD | MULTIPARAE X ± SD |
|---|---|---|
| women without clinical malaria; | 9.3±1.8 (n=119) | 9.6±1.6 (n=456) |
| women with clinical malaria; | 8.8±1.6 (n=54) | 9.1±2.1 (n=89) |

analysis of variance (complete model), an analysis was performed using an additive model. This analysis showed a significantly lower mean Hb concentration in nulliparae compared to multiparae (p=0.04).
Thus the analysis using the mean Hb concentration as a parameter indicates an effect of parity in contrast to the analysis using the WHO definition of anaemia (Table VI-8).

Since in this study prevalence of clinical malaria is higher in the first trimester of pregnancy (chapter VI-B) and anaemia is related to malaria the relation between clinical malaria, amenorrhea and mean Hb value was analysed. Figure VI-3 represents mean Hb values of visits with or without clinical malaria in relation to amenorrhea. The results show an almost consistent pattern of lower Hb values of women with clinical malaria as compared to those without clinical malaria (except for values at an amenorrhea of 20 weeks).
Statistical analysis of the data by a two-way analysis of variance (complete model) showed that the interaction hypothesis could not be rejected (p=0.79), i.e. the difference between the mean Hb concentration of women with or without

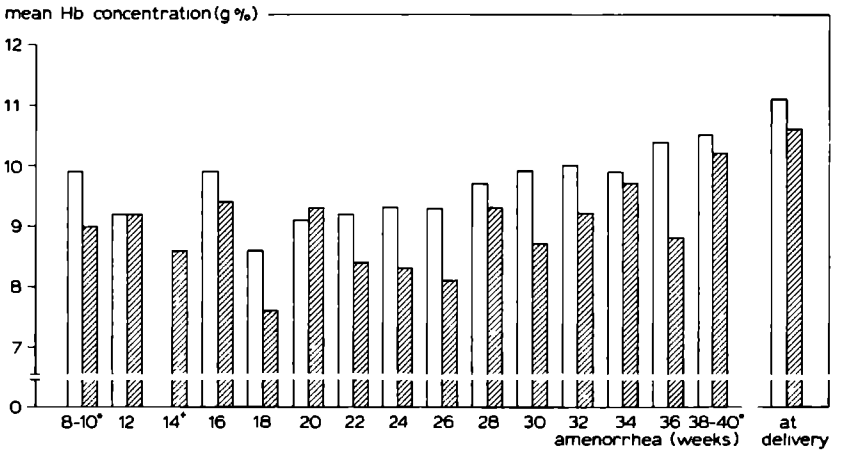



Fig. VI-3; Mean haemoglobin values of women with or without clinical malaria related to duration of amenorrhea: □ = mean Hb value of "non-malarious" women (n=574): ▨ = mean Hb value of women with clinical malaria (n=144): + women without clinical malaria were not present at this amenorrhea. * because of the small number of women available at these periods results were pooled.

clinical malaria could not be proven to depend on amenorrhea. A further analysis on the basis of an additive model (i.e. a two-way analysis model without interaction) showed a significantly higher mean value for the "non-malaria" group ($p<0.001$). These findings are in accordance with the analysis of Table VI-9. Furthermore, the hypothesis of an equal mean Hb concentration for all amenorrheal periods was rejected for "malaria positive" and "negative" women ($p<0.001$). Generally the mean Hb values of the visits before 28 weeks tend to be lower than after that period. Using a multiple comparison method each estimated mean Hb value of a given amenorrheal period for visits with or without clinical malaria was compared to all other Hb values on the basis of a two way additive analysis of variance with separate Student's t-tests. Introducing the same level of significance for the overall analysis of variance and the Student's t-tests according to the principle of Bonferroni we found a nearly significantly lower Hb concentration at 18 weeks compared to 32 and 38 weeks ($0.05<p<0.10$).

**Comparison of the haemoglobin concentrations at delivery and during pregnancy:**

The available data (n=170) on the Hb concentrations after delivery of those women (n=194) from the longitudinal study group who gave birth to their child in the hospital, were analysed as well. These women were divided into women with and without clinical malaria during their pregnancy. It has to be noticed that no account was given to the effect of a possible clinical malaria during labour nor was studied the relation between placental malaria and haemoglobin. The mean haemoglobin concentrations of these two groups after delivery were not significantly different (Table VI-10). For comparison these data were also included in figure VI-3.

Table VI-10
Mean haemoglobin concentration (g %) at delivery of women with and without clinical malaria during their pregnancy

| | n: | HAEMOGLOBIN X ± SD |
|---|---|---|
| women with clinical malaria; | 52 | 10.6±2.0 |
| women without clinical malaria; | 118 | 11.1±1.9 |

(Student's t-test: p=0.11)

Although women with clinical malaria during pregnancy, still have a lower Hb concentration at delivery, this difference was not significant any more in contrast to the significant difference observed during pregnancy (previous section).
The same conclusion is obtained when data are analysed in relation to parity. On the one hand the mean haemoglobin concentration of the women without recorded clinical malaria is slightly higher but on the other hand more multiparous women were present in this group (78%) compared to the malaria group (65%). Since multiparae with clinical malaria tend

to have higher mean haemoglobin concentrations the effect of a correction for parity may reduce further the difference between the "malarious" and "non-malarious" group.
The results in figure VI-3 suggest that the Hb concentration increases from week 28 towards delivery. Therefore Hb values of women with known amenorrhea (n=151), determined during their first visit at the antenatal clinic were compared to their values obtained at delivery in relation to malaria (Table VI-11).

Table VI-11
Comparison of the mean haemoglobin concentration measured at delivery with that observed at the first visit during pregnancy

| | HAEMOGLOBIN CONCENTRATION X ± SD (g %) | | |
|---|---|---|---|
| clinical malaria at first visit; | pregnancy | delivery | |
| yes (n=115); | 9.5±1.5 | 11.1±1.9 | p<0.001 |
| no (n=36); | 9.1±1.7 | 10.7±1.9 | p<0.001 |

(Student's t-test for paired samples)

Analysis of the results showed a significantly higher Hb level in both groups of women at delivery. Since the Hb concentration depended on amenorrhea, the data were further analysed with respect to the amenorrheal period corresponding to the first visit. The first visit data were classified according to the following trimesters: ≤16 weeks; 18-26 weeks, and ≥28 weeks. The results of this analysis are given in Table VI-12.
A significant difference is observed between first visit-haemoglobin values obtained during the second and third trimester and the haemoglobin concentration at delivery (Table VI-12).

Table VI-12
Comparison of the mean haemoglobin concentration measured at delivery with that observed at the first visit in relation the trimester

| first visit; | HAEMOGLOBIN CONCENTRATION (X ± SD) | | | |
|---|---|---|---|---|
| | **at delivery:** | **≤16wks:** | **diff:** | * |
| no clinical malaria;(n=8) | 11.1±2.4 | 9.3±1.7 | 1.8 | p=0.10 |
| clinical malaria;(n=6) | 11.6±1.7 | 10.3±1.0 | 1.3 | p=0.22 |
| | **at delivery:** | **16-26wks:** | **diff:** | * |
| no clinical malaria;(n=54) | 11.1±1.8 | 9.4±1.4 | 1.7 | p<0.001 |
| clinical malaria;(n=15) | 10.7±2.0 | 8.5±1.8 | 2.1 | p<0.001 |
| | **at delivery:** | **≥28wks:** | **diff:** | * |
| no clinical malaria;(n=53) | 11.2±1.9 | 9.6±1.6 | 1.6 | p<0.001 |
| clinical malaria;(n=15) | 11.5±1.9 | 9.3±1.6 | 1.2 | p<0.03 |

(*Student's t-test)

The differences between first visit Hb values during the first trimester and at delivery are not remarkably smaller than values in other trimesters. The absence of significance may be due to the small number of women in the study group, although inherent differences cannot be ruled out.
It is to be expected that vaginal bleeding tends to reduce the haemoglobin concentration. Consequently, it can be expected that the difference between values obtained immediately after delivery and during pregnancy are to a large extend influenced by the bleedings post partum. Hence, the difference between the Hb concentrations are in fact probably larger.

The results of the analysis on the changes of Hb concentration during pregnancy towards delivery (fig. VI-3 and Table VI-12) and at delivery, suggest that the effect of clinical malaria on Hb concentration is expressed most strongly after 16 weeks of amenorrhea and declines with progress of amenorrhea: the difference after delivery between women with and without clinical malaria during pregnancy is not significant.

2. Spleen enlargement during pregnancy

Spleen enlargement is one of the parameters that can be used for the characterization of malaria during pregnancy, although as mentioned already, the parameter as such is rather poor. In this analysis spleen enlargement at the first visit of "non-malarious" women and the last malaria visit of women with clinical malaria was analysed only.
The analysis of spleen enlargement in relation to clinical malaria is given on Table VI-13. The results show a signifi-

Table VI-13
Spleen enlargement in women with and without observed clinical malaria during pregnancy

| | CLINICAL MALARIA | |
|---|---|---|
| | no: | yes: |
| spleen not enlarged; | 538 (99%) | 164 (93%) |
| spleen enlarged; | 5 (1%) | 12 (7%) |
| total number; | 543 | 176 |

(Fisher's exact test; $p<0.001$)

cantly higher spleen rate in women with clinical malaria as compared to women without clinical malaria.
The chance to palpate an enlarged spleen in the group of malarious women is independent of parity (Table VI-14).

Table VI-14
Spleen enlargement in nulliparae and multiparae with clinical malaria during pregnancy

| | NULLIPARAE | MULTIPARAE |
|---|---|---|
| spleen not enlarged; | 58 (95%) | 105 (92%) |
| spleen enlarged; | 3 (5%) | 9 (8%) |
| total number; | 61 | 114 |

(Fisher's exact test; p=0.55)

According to these results, spleen enlargement is a symptom of malaria during pregnancy. The frequency of an enlarged spleen however is much smaller than that of malaria during pregnancy, which reduces the value of this parameter for diagnostic purpose.

To investigate whether a progressing amenorrhea, i.e. an increasing fundal height, interferes with the palpation of an enlarged spleen, spleen rates of "non-malarious" women at different amenorrheal periods were compared to spleen rates of women with clinical malaria. The results depicted in Table VI-15 suggest a higher chance to palpate an enlarged spleen in the first and second trimester than in the third trimester, though differences are not statistically significant either for "non-malaria" visits or for "malaria" visits (Table VI-15).

3. Fever in pregnant women

Fever (axillar temperature above 37.0°C; see chapter II), is an symptom of clinical malaria. Therefore, the frequency of fever present at a malaria visit was compared to that observed in women without documented clinical malaria during pregnancy (Table VI-16). The statistical analysis performed only on the first visit data, indicates a significantly

Table VI-15
Spleen rates in relation to women with or without clinical malaria

| amenorrhea | MALARIA VISIT $n_1/n_2$ | "NON-MALARIA" VISIT $n_1/n_2$ |
|---|---|---|
| ≤16 wks. | 3/26 (12%) | 1/44 (2%) |
| 18-26 wks. | 6/55 (11%) | 3/268(1%) |
| ≥28 wks. | 3/91 (3%) | 0/255(0%) |
| Fisher's exact test: | p=0.13 | p=0.13 |

$n_1$=number of enlarged spleens
$n_2$=total number of cases

Table VI-16
Frequency of fever among women with actual malaria or without documented clinical malaria during pregnancy

| | "NON-MALARIA" VISIT (non-malarious women) n: | MALARIA VISIT (malarious women) n: |
|---|---|---|
| no fever; | 491 (99%) | 100 (55%) |
| fever; | 7 (1%) | 82 (45%) |
| total number of visits; | 498 | 182 |

(Fisher's exact test; p<0.001)

higher frequency of fever at visits with clinical malaria, and this frequency of fever was independent of parity (Table VI-17).

Table VI-17
Frequency of fever among women with clinical malaria in relation to parity

| | NULLIPARAE n: | MULTIPARAE n: |
|---|---|---|
| no fever; | 35 (55%) | 65 (56%) |
| fever; | 29 (45%) | 51 (44%) |
| total number of visits; | 64 | 116 |

(Fisher's exact test; p>0.10)

## 4. Characteristics of the newborn in relation to clinical malaria during pregnancy

**Birth weight in relation to clinical malaria and parity:**

From the women included in the longitudinal study 194 delivered in the hospital and gave birth to 196 children: 103 females and 93 males. The average birth weight in this group was 2942 ± 541 g. Since parity as well as clinical malaria affect birth weight (chapter I), and the frequency of clinical malaria depends on parity (chapter VI-B), the influences of parity and clinical malaria on birth weight were studied together. Twin births and still births and children of women with unknown parity were excluded from analysis. The remaining 161 women were divided into groups of nulliparae and multiparae with or without clinical malaria. For each group the average birth weight of their newborns was calculated. The results are given in Table VI-18 and analysed by a two-way analysis of variance (complete model).

The interaction hypothesis, that the difference in mean birth weight between babies from nulliparae and multiparae was the same for malaria mothers and "non-malaria" mothers could not be rejected (p=0.72). Additionally, no significant

Table VI-18
Mean birth weight in relation to parity and clinical malaria of the mother

| | "NON-MALARIA"-MOTHER | | MALARIA-MOTHER | |
|---|---|---|---|---|
| | n: | X ± SD | n: | X ± SD |
| nulliparae; | 23 | 2778±431 g | 17 | 2818±434 g |
| multiparae; | 92 | 3027±475 g | 29 | 3002±497 g |

difference was found between the mean birth weight of babies born out of malarious and "non-malarious" women (p=0.94), but a significantly greater mean birth weight was found for babies of multiparae relative to nulliparae (p=0.02).
Thus, the analysis shows a significant increase in birth weight in multiparae, but no reduction in birth weight due to clinical malaria during pregnancy.

**Prematurity:**

A living newborn infant with a birth weight of 2500 gram or less is taken as a premature live-birth according to the

Table VI-19
Number of premature live born infants (according to the WHO definition) related to clinical malaria for nulliparae and multiparae

| | TOTAL | NULLIPARAE | MULTIPARAE |
|---|---|---|---|
| | $n_1/n_2$ | $n_1/n_2$ | $n_1/n_2$ |
| no clinical malaria; | 14/115 (12%) | 4/23 (17%) | 10/92 (11%) |
| clinical malaria; | 9/46 (20%) | 3/17 (18%) | 6/29 (21%) |
| Fisher's exact test: | p=0.22 | p=1.0 | p=0.21 |

$n_1$=number of premature born infants
$n_2$=total number of cases

definition recommended by the World Health Organization (1950). After exclusion of twin births the number of premature live-born infants was scored for nulliparae and multiparae and in relation to clinical malaria during pregnancy. The analysis with regard to the effect of clinical malaria on prematurity is given on Table VI-19 and with regard to the effect of parity on Table VI-20. The results indicate that in this study group neither clinical malaria during pregnancy nor parity are significantly related to prematurity.

Table VI-20
Premature live born infants (according to the WHO definition) in relation to parity and clinical malaria of the mother

| | TOTAL $n_1/n_2$ | "NO-MALARIA" $n_1/n_2$ | MALARIA $n_1/n_2$ |
|---|---|---|---|
| nulliparae; | 7/40 (18%) | 4/23 (17%) | 3/17 (18%) |
| multiparae; | 16/121(13%) | 10/92 (11%) | 6/29 (21%) |
| Fisher's exact test: | p=0.60 | p=0.47 | p=1.0 |

$n_1$=number of premature born infants
$n_2$=total number of cases

**Condition of the newborn:**

The health condition of the newborn was expressed by an Apgar score (for details see chapter II) determined 1 and 5 minutes after birth. The newborns were divided in groups related to both parity and presence or absence of recorded clinical malaria during pregnancy of the mother (Table VI-21 and VI-22). Available data (n=170) of paired Apgar scores were analysed only.

Table VI-21

Mean Apgar score at 1 and 5 minutes of babies from mothers with or without clinical malaria, and in relation to parity

| | "NON-MALARIOUS" | MALARIOUS MOTHER | |
|---|---|---|---|
| all mothers; | (n=121) | (n=49) | * |
| Apgar score 1 min: | 8.1 | 8.4 | p=0.88 |
| Apgar score 5 min: | 9.4 | 9.4 | p=0.78 |
| nulliparae; | (n=25) | (n=17) | * |
| Apgar score 1 min: | 7.4 | 8.6 | p=0.09 |
| Apgar score 5 min: | 8.8 | 9.8 | p=0.20 |
| multiparae; | (n=96) | (n=32) | * |
| Apgar score 1 min: | 8.3 | 8.3 | p=0.45 |
| Apgar score 5 min: | 9.5 | 9.3 | p=0.26 |

(*Wilcoxon test)

Table VI-22

Mean apgar score at 1 and 5 minutes of babies from nulliparae or multiparae, and in relation to clinical malaria

| | NULLIPARAE | MULTIPARAE | |
|---|---|---|---|
| all mothers; | (n=42) | (n=128) | * |
| Apgar score 1 min: | 7.9 | 8.3 | p=0.14 |
| Apgar score 5 min: | 9.2 | 9.4 | p=0.07 |
| "non-malarious" mothers; | (n=25) | (n=96) | * |
| Apgar score 1 min: | 7.4 | 8.3 | p=0.02 |
| Apgar score 5 min: | 8.8 | 9.5 | p=0.01 |
| malarious mothers; | (n=17) | (n=32) | * |
| Apgar score 1 min: | 8.5 | 8.3 | p=0.54 |
| Apgar score 5 min: | 9.8 | 9.3 | p=0.70 |

(*Wilcoxon test)

Results depicted on table VI-21 show no significant difference between the Apgar score of infants born from women with or without clinical malaria during pregnancy, neither at 1 nor at 5 minutes after delivery. In addition this result was independent of the parity of the mother, although there was a slight indication (p=0.09) for a higher Apgar score in case of nulliparae with clinical malaria during pregnancy. Thus, differences in Apgar score are apparently not related to clinical malaria in this study group.

With regard to parity significantly higher Apgar scores were recorded in newborns from multiparae relative to nulliparae in mothers without recorded malaria during pregnancy (Table VI-22). This phenomenon is not found in mothers with clinical malaria during pregnancy, which seems to be due to higher Apgar scores in nulliparae with clinical malaria in comparison to nulliparae without recorded clinical malaria during pregnancy.

The deathrate due to still birth and neonatal death (Apgar score 0 at 5 minutes) was 5%. Further analysis (Fisher exact test) did not show a significant difference in probability of neonatal death between babies of mothers with or without a recorded attack of clinical malaria, neither in the group as a whole (p=1.0), nor in the nulliparous (p=0.51) and multiparous subgroups (p=0.64).
Analogous results were found for the relation between neonatal death and parity in the group as a whole (p=1.0) or in the subgroups with (p=0.45) or without (p=0.60) recorded clinical malaria.
Parity is apparently more important for the neonatal condition than clinical malaria of the mother during their pregnancy. The finding of higher Apgar scores of babies born from nulliparae with clinical malaria during pregnancy was surprising and no reason could be found for this phenomenon.

## E: SERUM CONCENTRATION OF TOTAL CORTISOL IN PREGNANT WOMEN WITHOUT RECORDED CLINICAL MALARIA

### 1. Cortisol levels related to amenorrhea

The total serum cortisol values were determined in the available sera of women from the longitudinal study group who did not report, or exhibit clinical malaria during pregnancy (n=527). Due to loss of samples during preservation, transport or analysis, and unknown amenorrhea, only 527 samples of sera were available out of 601 sampled women.

Only the data of one sampling visit were included in this analysis. When a woman visited the antenatal clinic several times during pregnancy, arbitrarily only the cortisol value of the last sampling visit was plotted. The data were plotted against amenorrhea as shown in figure VI-4.

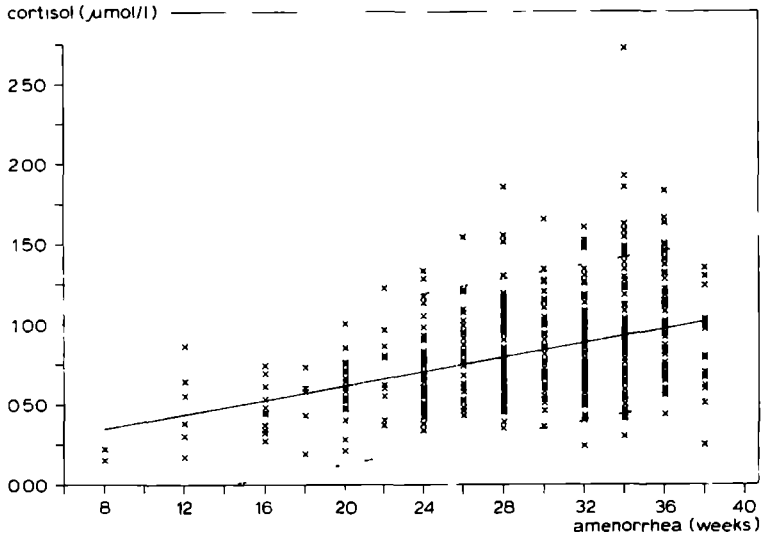



Fig. VI-4; Serum concentration of total cortisol in pregnant women without recorded clinical malaria (n=527); regression line and 90% prediction curves are given.

Results indicate a steady increase of the total cortisol concentration with increasing amenorrhea. Statistical analysis proves that the mean cortisol concentration for each

amenorrheal period was not the same (one way analysis of variance; $p<0.001$) and the hypothesis of a linear relation between mean cortisol and amenorrhea could not be rejected (F-test; $p>0.10$). The regression relation was expressed by $y=0.17+0.022x$, where y=mean cortisol (µmol/L) and x=amenorrhea (weeks).

In the group of women who were sampled twice (n=204), separate regression lines were calculated for cortisol values from first and second visits. The results show a lower value for the slope of the regression line from second ($y=0.48+0.014x$) as compared to first visit data ($y=0.21+0.021x$). These results may indicate a more rapid increase in cortisol concentrations in the first part of pregnancy which levels off in the second part.

## 2. Cortisol related to parity

Women without recorded clinical malaria during pregnancy were subdivided in nulliparae and multiparae and the data of the total serum cortisol values (last visit data in appropriate cases) were plotted in relation to amenorrhea. The results are given in figure VI-5.

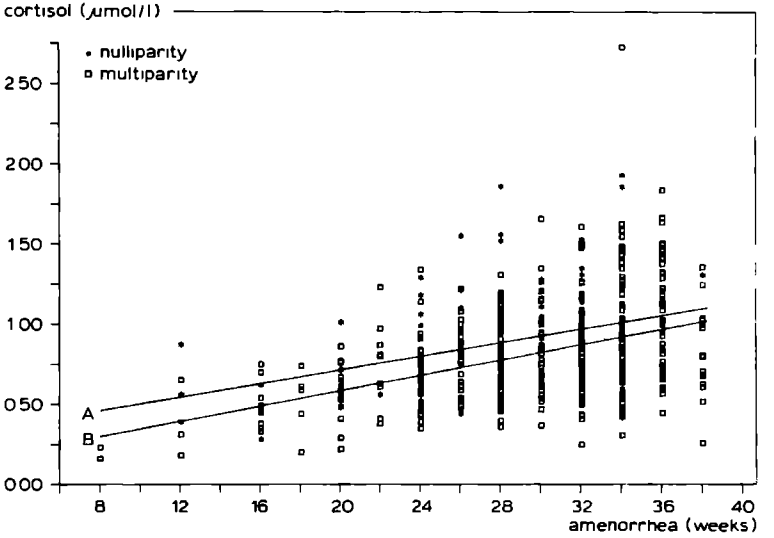



**Fig. VI-5; Serum concentration of total cortisol in pregnant women without recorded clinical malaria; comparison between nulliparae (n=105) and multiparae (n=422). Values of total cortisol in nulliparae (*) and multiparae (□) are plotted and regression lines of nulliparae (A) and multiparae (B) are given.**

The regression lines were y=0.28+0.021x for the nulliparae (n=105), and y=0.095+0.024x for the multiparae (n=422).The slopes of both lines were significantly different from zero ($p<0.001$).

Comparison of regression lines indicates a difference between cortisol levels of nulliparous and multiparous "non-malarious" women (fig. VI-5). Due to small numbers, data from week 10 and 40 were pooled with those of week 8 and 38 respectively, before a two-way analysis of variance with the factors amenorrhea and parity was carried out. The hypothesis that a possible difference between nulliparity and multiparity did not depend on amenorrhea could not be rejected (interaction test: $p=0.20$). Analysis on the basis of a two-way analysis of variance model without interaction, indicated significantly different cortisol levels in nulliparae relative to multiparae ($p=0.001$).

Thus, among women without recorded clinical malaria during pregnancy nulliparae have a significantly higher concentration of total cortisol in their serum than multiparae.

3. Cortisol related to age

Since parity is related to age and the cortisol concentration increases during pregnancy, analysis of the relation between age and cortisol concentration in pregnant women without recorded clinical malaria must also take into account the factors parity and amenorrhea. Only nulliparae and multiparae with known age (n=519) were analysed separately. Both groups were subdivided into three age classes: 15 to 24 years, 25 to 34 years and 35 to 45 years. In case of repeated visits to the clinic only the last visit data were included in this analysis. In the subgroup of nulliparae no woman was present in the age class 35 to 45 years. Amenorrhea was added to this analysis as a co-variable factor, i.e. a linear relation between amenorrhea and cortisol concentration was presumed, being the same for each parity and age class. Therefore, the mean cortisol concentration of each subgroup was recalculated on the basis of an amenorrhea

of 29.6 weeks (mean of this study group). The results are given on Table VI-23.

Table VI-23
Adjusted mean cortisol concentration (µmol/L) in relation to age in pregnant women without recorded clinical malaria (amenorrhea 29.6 weeks)

| age class: | Nulliparae; | | Multiparae; | |
|---|---|---|---|---|
| years: | n: | mean | n: | mean |
| 15≤24 | 94 | 0.91 | 133 | 0.84 |
| 25≤34 | 9 | 0.86 | 197 | 0.80 |
| 35≤45 | | | 86 | 0.78 |

The hypothesis that there were no differences between the age classes was tested by means of an one-way analysis of covariance. The decrease in mean total cortisol with increasing age was neither significant for nulliparae ($p=0.68$) nor for multiparae ($p=0.23$).

## F: SERUM CONCENTRATION OF TOTAL CORTISOL IN PREGNANT WOMEN WITH CLINICAL MALARIA

### 1. Cortisol levels related to amenorrhea

Serum concentration of total cortisol of the available sera from pregnant women with actual clinical malaria (n=152) was plotted against amenorrhea (fig. VI-6). When a woman exhibited clinical malaria at repeated visits, only the cortisol value of the arbitrarily chosen first malaria visit was plotted in this figure.
Regression analysis of the data (fig. VI-6) suggest a linear relation between cortisol concentration and amenorrhea which

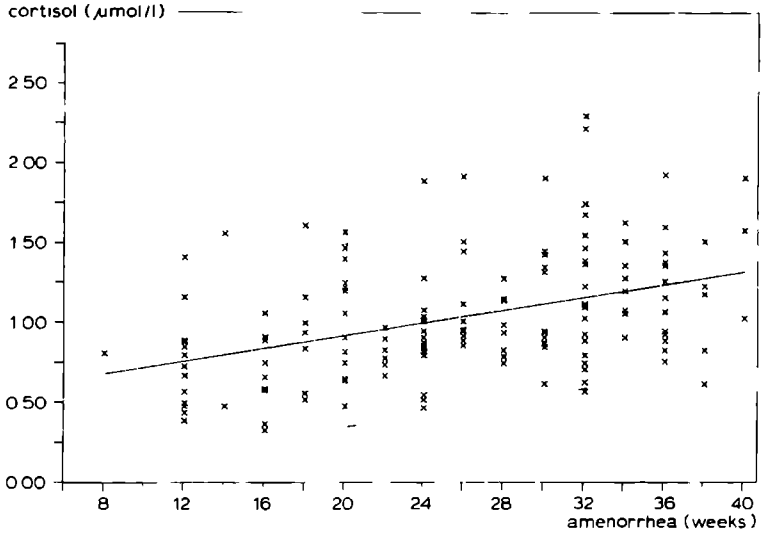



Fig. VI-6; Serum concentration of total cortisol in pregnant women with clinical malaria (n=152). Regression line and 90% prediction curves are given.

is described by y=0.51+0.02x (y=cortisol concentration µmol/L and x=amenorrhea in weeks). This result is comparable to that obtained for pregnant women without recorded clinical malaria. Direct comparison of these data (fig. VI-7)

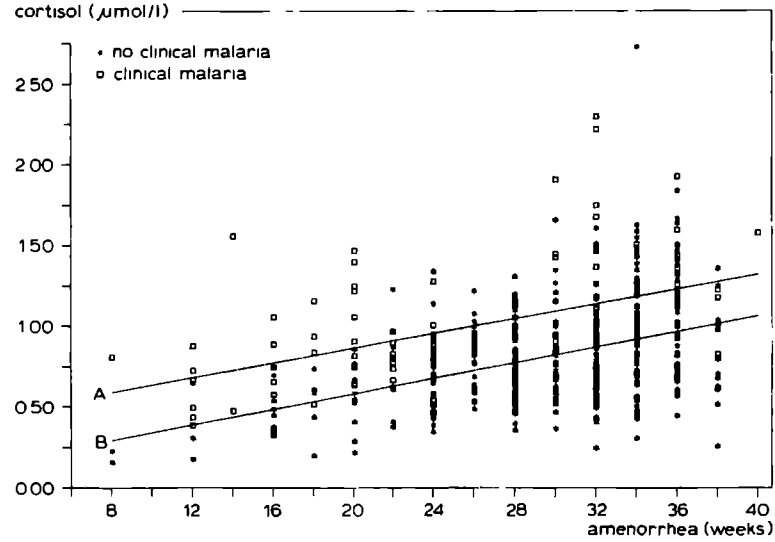



Fig. VI-7; Serum concentration of total cortisol in pregnant women with and without clinical malaria. Cortisol values of malaria (□) and "non-malaria"visits (*) are plotted. Regression lines of "non-malarious" women (A) and women with clinical malaria (B) are given.

reveals higher cortisol concentrations in the group with clinical malaria.

A two-way analysis of variance with factors amenorrhea and clinical malaria indicated that this difference was independent of amenorrhea (interaction test: p=0.79). On the basis of a two-way analysis of variance model without interaction however this difference was shown to be significant (p<0.001).

Thus, women with actual clinical malaria experience higher cortisol levels independent of parity and amenorrhea.

## 2. Cortisol levels related to parity

The group of women with clinical malaria described above was subdivided according to parity. Serum concentration of total cortisol at the first malaria visit of nulliparae (n=57) and multiparae (n=95) were plotted against amenorrhea (Fig.VI-8). The estimated regression line for the cortisol

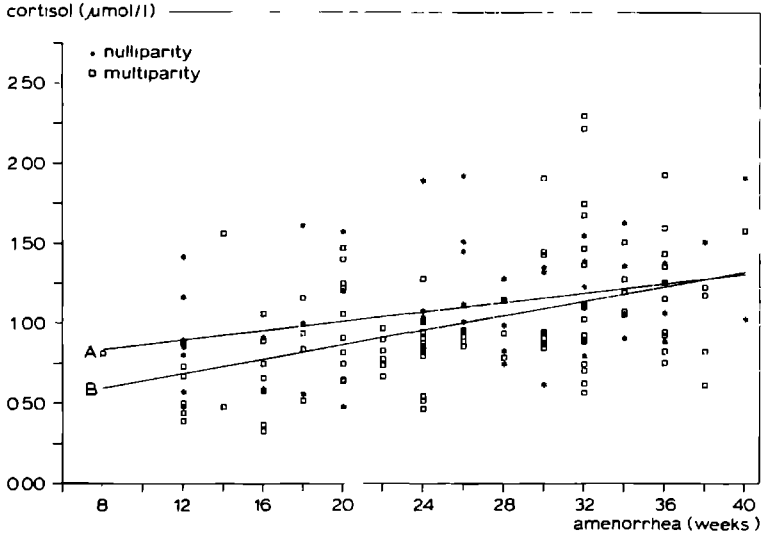



Fig. VI-8; Serum concentration of total cortisol in pregnant women with clinical malaria; comparison between nulliparae (n=57) and multiparae (n=95). Values of total cortisol in nulliparae (*) and multiparae (□) are plotted, and regression lines of nulliparae (A) and multiparae (B) are given.

data from nulliparae was y=0.71+0.015x and for those of multiparae was y=0.40+0.023x. The slopes of both lines were significantly different from zero (p=0.01 and $p < 0.001$). Since cortisol levels of nulliparae differed significantly from multiparae in the absence of recorded clinical malaria, a statistical analysis of the difference between nulliparae and multiparae with clinical malaria was also performed.
The two-way analysis of variance with the factor amenorrhea and parity indicated that the hypothesis that a possible difference between nulliparae and multiparae did not depend on the amenorrhea could not be rejected (interaction test: p=0.53). The two-way analysis of variance model without interaction gave an indication for a higher cortisol level in the nulliparae (p=0.09). The regression lines in figure VI-8, cross over at the end of pregnancy. This might suggest an amenorrhea depended difference between nulliparae and multiparae. The results of the interaction test (p=0.53, see above), however indicate that this difference is independed of amenorrhea despite the suggestion of the illustrated crossing over.

As described above the serum concentrations of total cortisol of pregnant women with clinical malaria were significantly higher than cortisol concentrations of pregnant women without clinical malaria (fig. VI-7). Parity was not considered in this analysis though it appeared to have a significant effect of its own in the group without recorded clinical malaria. Comparison of the data from pregnant women with or without clinical malaria subdivided in nulliparae and multiparae is made in fig. VI-9 and VI-10 respectively.
The two-way analysis of variance with factors clinical malaria and amenorrhea indicated that the differences in cortisol values of pregnant women with or without clinical malaria were significant, both in the group of nulliparae ($p < 0.001$) as well as in the group of multiparae ($p < 0.001$). This difference, however, did not depend on the amenorrhea (nulliparae p=0.59; multiparae p=0.62).
The decrease in cortisol concentration in relation to parity

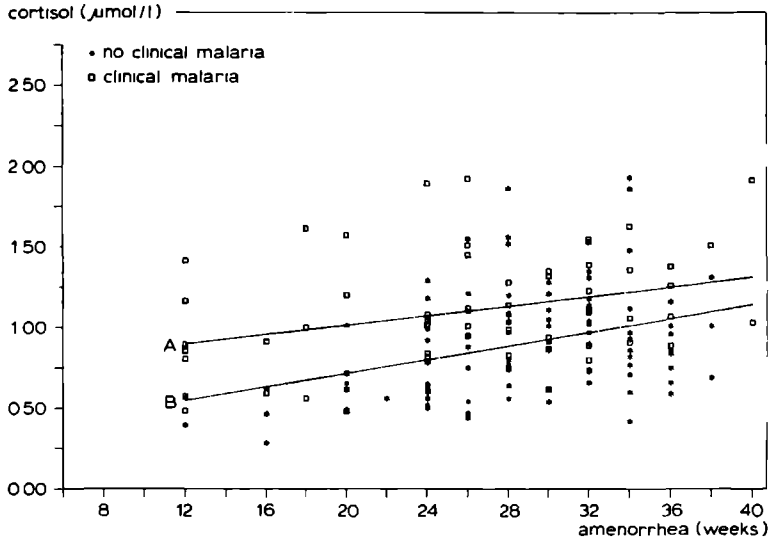



Fig. VI-9; Serum concentration of total cortisol in pregnant nulliparae; comparison between women with and without recorded clinical malaria. Values of total cortisol in "non-malaria" visits (*) of "non-malarious" women and malaria visits (□) of women with a recorded clinical malaria attack are plotted. Regression lines of malarious (A) and "non-malarious" women (B) are given.

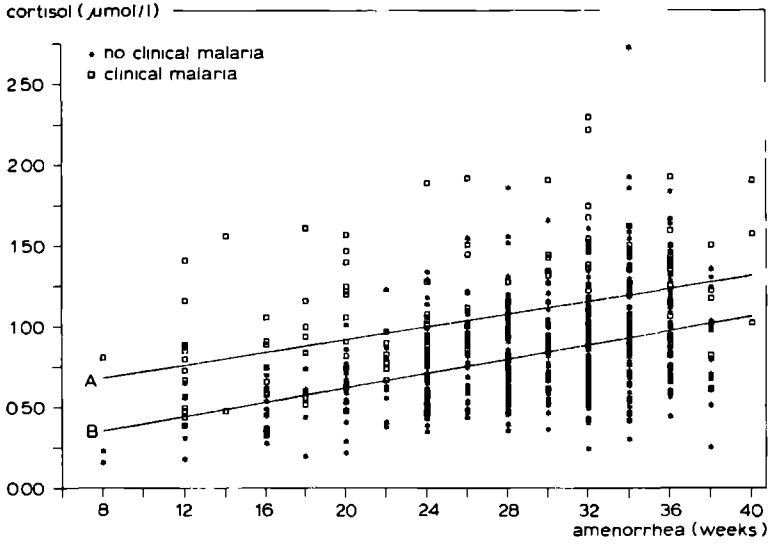



Fig. VI-10; Serum concentrations of total cortisol in pregnant multiparae; comparison between women with and without recorded clinical malaria. Values of total cortisol in "non-malaria" visits (*) of "non-malarious" women and at malaria visits (□) of women with a recorded clinical malaria attack are plotted. Regression lines of malarious (A) and non-malarious women (B) are given.

in women without recorded clinical malaria (fig. VI-5) is substantially smaller than the increase in cortisol concentration in relation to clinical malaria both in nulli- and multiparae (compare fig. VI-9 and 10)

3. Cortisol and loss of malaria immunity during pregnancy

If serum cortisol is involved in the regulation of immune reactivity, the observation that nulliparae have higher cortisol levels than multiparae may be relevant. Higher cortisol levels may be related to a higher frequency of clinical malaria.

For the analysis of a possible relation between cortisol, parity and clinical malaria, women were divided in groups according to the presence or absence of clinical malaria observed during pregnancy and subdivided in nulliparae and multiparae. The corresponding cortisol levels were recalculated, on the basis of a linear relationship between cortisol values and amenorrhea to the value at 29 weeks amenorrhea (average of this study group). The data are presented on Table VI-24. The interaction hypothesis tested by a

Table VI-24

Mean serum concentration of total cortisol (µmol/L) recalculated for a mean amenorrhea of 29 weeks

| | | n: | MEAN CORTISOL: |
|---|---|---|---|
| no clinical malaria: | nulliparae; | 105 | 0.89 |
| | multiparae; | 422 | 0.79 |
| clinical malaria: | nulliparae; | 57 | 1.15 |
| | multiparae; | 95 | 1.07 |

two-way analysis of variance with amenorrhea as a covariable indicated that the hypothesis that the difference between cortisol values of nulliparae and multiparae are the same in

pregnant mothers with or without recorded clinical malaria could not be rejected ($p=0.82$).
Cortisol levels were significantly different in the pregnant women with recorded clinical malaria relative to those without recorded clinical malaria ($p<0.001$), as well as in the group of nulliparae relative to multiparae ($p<0.002$).

In a further analysis it was tested whether fever and anaemia could be used to "purify" the analytical groups. For this purpose only those women who presented with fever were selected from the group of women with clinical malaria, whereas the control women with a haemoglobin concentration higher than 9.5% (see table VI-9), and without fever were selected for analysis. The results of a variance and a co-variance analysis performed like that described in table VI-24 showed the same "malaria effect" (average difference of 0.28 µmol/L cortisol) and "parity effect" (average difference of 0.09 µmol/L cortisol). These results apparently indicate that fever and anaemia are relatively unimportant parameters in these analyses.
The result of only one sampling visit from each woman was included in the analysis: the last visit in case of a woman without a recorded malaria attack and the first malaria visit of a woman with clinical malaria.
To characterize the relation between serum cortisol and clinical malaria further the rate of increase in serum cortisol was analyzed. The observed linear increase in the serum concentration of total cortisol relative to amenorrhea allows calculation of an increase rate per day. This cortisol increase rate was separately calculated from the data of each woman who visited the clinic and was sampled more than once. One group consisted of women without recorded clinical malaria and in the other group the increase rate was calculated from the data obtained at a malaria visit combined with the data obtained of the previous non-malaria visit. Both groups were subdivided in nulliparae and multiparae (Table VI-25).

Table VI-25
Mean increase rate ($\mu mol \times 10^3/L/day \pm SD$) of total cortisol in pregnant nulliparae and multiparae with or without recorded clinical malaria

| | CLINICAL MALARIA yes: | no: | Stud.t-test: |
|---|---|---|---|
| nulliparae; | 4.0±5.3 (n=33) | 12.5±10.6 (n=9) | p=0.002 |
| multiparae; | 3.0±7.1 (n=172) | 3.5±11.4 (n=11) | p=0.84 |

Nulliparae who develop clinical malaria have a significantly greater daily increase of total cortisol compared to nulliparae without.
The cortisol increase rate in multiparae that develop clinical malaria however, is not increased in comparison to multiparae without a record of clinical malaria. Significantly higher increase rates of serum concentrations of total cortisol may have been caused by increased concentrations of total serum cortisol during clinical malaria independent of changes in the pre-malaria period. It is therefore interesting to analyse cortisol increase rates in non-malarious periods preceding clinical malaria. The material included only three cases and their data are compared to the cortisol increase rate in women without recorded clinical malaria during pregnancy (Table VI-26).

The data suggest that the cortisol increase rate in women that develop clinical malaria during pregnancy is higher already before the recrudescence. However, the difference is not significant (Student's t-test: p=0.08; Welch test: p=0.64).
It should be noted that most women who developed clinical malaria were not seen twice before this happened.

Table VI-26

Mean cortisol increase rate ($\mu mol \times 10^3/L/day \pm SD$) between two non-malaria visits of women with or without clinical malaria during pregnancy

| | n: | X ± SD |
|---|---|---|
| no clinical malaria; | 205 | 3.2± 6.8 |
| clinical malaria; | 3 | 10.6±23.7 |

In another approach the cortisol level of women with clinical malaria during pregnancy at their visit preceding clinical malaria is compared to the level of women without recorded clinical malaria, being determined at about the same amenorrheal period. Therefore the data of the second sampling visit of women without recorded clinical malaria, *and of the last non-malaria visit preceding clinical malaria* were analyzed in a two-way analysis of covariance with amenorrhea as a covariable. For this analysis a linear dependence of cortisol concentration on amenorrhea is assumed for both groups. The mean cortisol concentrations recalculated for the same amenorrhea are shown in table VI-27.

Table VI-27

Recalculated mean cortisol concentration (µmol/L) of non-malaria visits for nulliparae and multiparae with or without clinical malaria

| | | n: | MEAN: | amenorrhea: |
|---|---|---|---|---|
| nulliparae | no clinical malaria; | 107 | 0.83 | 26 weeks |
| | clinical malaria; | 10 | 0.84 | |
| multiparae | no clinical malaria; | 418 | 0.76 | 28 weeks |
| | clinical malaria; | 14 | 0.86 | |

The analysis neither showed a significant difference in nulliparae ($p=0.94$) nor in multiparae ($p=0.20$).
The correctness of the above (table VI-27) included assumption of a linear increase of total serum cortisol levels towards a recrudescence was analysed further. For this purpose the cortisol data from the non-malaria visits of women who suffered from clinical malaria later on during pregnancy, were classified as higher or lower than the mean value obtained from women without recorded clinical malaria. In nulliparae it appeared that the higher values were sampled with an average period of 31 days before recorded clinical malaria and the lower values with an average of 62 days.
For multiparae these figures were 40 days for the higher and 58 days for the lower values.
These data seem to indicate that the increase towards clinical malaria is not linear for women who develop clinical malaria. In this case an increasing period between the two sampling visits reduces the rate of increase and thereby the chance to detect significant differences.
The data used in this analyses contain only one sampling point before the malaria visit, and only a limited number of cases is available. More sampling points are needed for the characterization of the period of cortisol increase towards clinical malaria.

So far the results (Table VI-26) indicate that it is very difficult to use changes in the concentration of total cortisol to predict development of clinical recrudescences during pregnancy, due to the small number of women.
Multiparae with clinical malaria have higher cortisol values (Table VI-24 and fig. VI-10), but the daily increase of the cortisol concentration during the preceding period was not different from that found in multiparae without clinical malaria (Table VI-25). Thus multiparae with clinical malaria later during pregnancy apparently have higher cortisol values throughout. The higher cortisol concentration of the non-malaria visits in multiparae with clinical malaria (Table VI-27) may support this view.

In nulliparae in contrast to multiparae the cortisol level before recorded clinical malaria is the same as that in nulliparae without clinical malaria. Yet the cortisol level in nulliparae with clinical malaria as well as the rate of increase towards the clinical recrudescence are higher than in nulliparae without a record of clinical malaria. This suggests a different mechanism in multiparae and nulliparae with regard to changes in cortisol level in relation to clinical malaria.

When cortisol levels are elevated due to clinical malaria the question is whether the level will return to normal after cure. The values observed during a visit with clinical malaria, and a subsequent non-malaria visit, which was not followed later on by another visit with clinical malaria were not significantly different (mean difference 0.03 µmol/L; n=35; p=0.65; Student's t-test for paired samples), neither for nulliparae (p=0.85; mean difference 0.02 µmol/L), nor for multiparae (p=0.54; mean difference 0.06 µmol/L). This observation prompted the analysis of the difference between the cortisol concentration of non-malaria visits after a visit with clinical malaria, and the values of non-malaria visits of women without a record of clinical malaria during pregnancy.
The non-malaria visits after clinical malaria took place between 20 and 38 weeks amenorrhea (Table VI-28). Thus, only cortisol values from visits of 20 weeks of amenorrhea or later from women without recorded clinical malaria were compared to those of non-malaria visits after a visit with

Table VI-28
Frequency distribution of non-malaria visits after a visit with clinical malaria

| AMENORRHEA: | 20 | 22 | 24 | 26 | 28 | 30 | 32 | 34 | 36 | 38 | weeks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| number of visits: | 2 | 0 | 4 | 1 | 2 | 5 | 7 | 4 | 6 | 1 | |

clinical malaria. The cortisol values were recalculated to the value of 30 weeks amenorrhea (average of these subgroups) on the basis of a linear relationship between cortisol values and amenorrhea.

Table VI-29
Recalculated mean concentration of total cortisol (µmol/L) for an amenorrhea of 30 weeks; concentrations at non-malaria visits of non-malarious women are compared to those at non-malaria visits after a visit with clinical malaria.

| | n: | MEAN CORTISOL: |
|---|---|---|
| non-malarious women | 501 | 0.85 |
| malarious women | 32 | 0.94 |

The data are presented in Table VI-29. One-way analysis of covariance with amenorrhea as a covariable, pointed to a higher total cortisol concentration of the non-malaria visits following a visit with clinical malaria ($p=0.10$).
The mean difference of days between the visit with clinical malaria and the subsequent non-malaria visit was 65 days. This long interval makes it unlikely that the high cortisol concentration found at the visit after the visit with clinical malaria was caused by malaria itself.
This finding may indicate that women who develop a clinical malaria recrudescence during pregnancy have a higher cortisol level throughout their pregnancy compared to women without loss of malaria immunity.

Parasite density may be used to characterize the severity of the disease which may in turn affect the cortisol level. Therefore, cortisol levels were compared to the parasite density index (PPDI) at the first malaria visit and in relation to amenorrhea. The analysis comprised the data of 135 women.

The analysis gave a value of 0.10 for the Pearson correlation between cortisol and PPDI, and a value of 0.14 for the partial correlation given the amenorrhea. Both correlations were not significantly different from zero (resp. p=0.25 and p=0.11). Thus no relation between parasite density and cortisol level could be detected in this study group.

## G: THE FREE FRACTION OF CORTISOL IN SERUM OF PREGNANT WOMEN

Since free cortisol is considered to be the biologically active fraction for some of the effects elicited by cortisol (see chapter I-E), it is important whether pregnancy dependent changes in the serum concentration of total cortisol are also shown by free cortisol.
The fraction of free cortisol was determined as described in chapter II (material and methods), in sera of 63 women taken at random from the longitudinal study group, and in sera of 5 non-pregnant women from the non-pregnant reference group.
It was presumed that the influence of amenorrhea, parity, and clinical malaria on the fraction of free cortisol was not different from that observed on total cortisol. Since this hypothesis was tested with the same analytical model as has been used for total cortisol, the fraction of free cortisol rather than the concentration of free cortisol was used in the following analysis.
Data available on total cortisol is much larger than on free cortisol. Hence it is more appropiate to ask whether free cortisol changes relative to total cortisol. The scatter in the individual data would complicate the ascertainment of statistical significance when the analysis would be based on an anlysis of the effect of clinical malaria, parity, or amenorrhea on the free concentration of cortisol rather than on changes in the free fraction in relation to changes in total cortisol. Moreover, the hypothesis that free cortisol changes relative to changes in total cortisol is more obvious, and considering the normal physiological changes

(e.g. diurnal rhythm) the free fraction is a more stable parameter.
Pilot statistical analyses using either the free fraction or the free concentration have supported this reasoning.

1. The free fraction of cortisol in serum of pregnant women compared to non-pregnant women

Since total cortisol concentration in serum of pregnant women is higher than in serum of non-pregnant women, it is important to know whether this pregnancy induced increase is also reflected in free cortisol.
The free fraction of cortisol was determined in sera of 5 non-pregnant women (mean age: 25±5 years), and the results compared to those from sera of 5 pregnant women (mean age: 29± 6 years), obtained at two sampling visits during their pregnancy. The first sampling visit took place at a mean amenorrhea of 20.4±4.8 weeks and the second at 30.4±3.9 weeks.
The serum fraction of free cortisol in non-pregnant women was 5.6%±0.6% (mean±SD) and that of pregnant women was 6.5%±1.3% (first visits) and 6.1%±0.7% (second visits). According to these data the mean values of free fraction of cortisol are higher in sera of pregnant relative to non-pregnant women, but the differences were neither significant for the first nor for the second visit (Student's t-test: $p>0.05$ in both cases).
In view of the higher serum concentration of total cortisol in pregnant relative to non-pregnant women (chapter IV and V,VI), this seems to indicate a higher serum concentration of free cortisol as well. The possible effect of the amenorrhea on free cortisol will be analysed in more detail in the following section. The data on free cortisol in this section were collected within one analytical run. Due to methodological differences these values cannot be compared to those described in the following sections (see also chapter II-D).

2. The free fraction of serum cortisol related to amenorrhea

In view of the changes in serum concentration of total

cortisol during pregnancy in relation to amenorrhea, changes in the free fraction of cortisol in relation to amenorrhea were analysed as well. Free fraction of cortisol was not measured in all women of study group I (longitudinal study group), but only in a group of women selected at random (n=63): 29 women without a record of clinical malaria at two sampling visits, and 31 women at their malaria as well as at their "non-malaria" visits (Table VI-30).
Parity was not included as a variable in the analysis of these data since differences between nulliparae and multiparae for the corresponding amenorrhea were not significant (p=0.18; see section VI-G3).
One-way analysis of variance of the free fraction of serum cortisol observed at different stages of pregnancy in relation to the first or second sampling visit in women without recorded clinical malaria, did not reveal significant differences (first visit: p=0.34 and second visit: p=0.55). Therefore, the results of the two sampling visits were summarized on Table VI-30, although comparison of the free fractions between the two visits of each woman indicated a very slight increase: 0.04% pro week (Student's t-test for paired samples: p=0.06).

The same analysis applied to the free fraction of serum cortisol of women without recorded clinical malaria, was used for analysis of the free fraction at visits with malaria (table VI-30). Again, the influence of parity was neglected (see following section G-3). Despite high values for the free fraction of serum cortisol (9.1% and 14.3%) in two women with clinical malaria at 12 weeks amenorrhea, the mean value of this group did not differ significantly from the values observed at different stages of pregnancy (p=0.17).
Thus, the data in this section indicate that there is no significant change in the fraction of free cortisol between 12 and 38 weeks of amenorrhea.
In view of the significant increase of total cortisol during pregnancy, this indicates that the serum concentration of

free cortisol rises during gestation independent of clinical malaria during pregnancy.

Table VI-30
The mean free fraction of serum cortisol of women with or without recorded clinical malaria in relation to amenorrhea

| | RECORDED CLINICAL MALARIA | | | |
|---|---|---|---|---|
| | YES: | | NO: | |
| amenorrhea: (weeks) | n: | free cortisol X ± SD (%) | n: | free cortisol X ± SD (%) |
| 12 | - | - | 4 | 8.4±4.5 |
| 16 | 1 | 4.6 | 3 | 3.3±0.8 |
| 18 | - | - | 3 | 4.6±0.3 |
| 20 | 9 | 4.1±0.5 | 2 | 4.3 |
| 22 | 2 | 3.9 | - | - |
| 24 | 7 | 4.0±0.8 | - | - |
| 26 | 5 | 4.7±1.0 | 3 | 4.2±0.3 |
| 28 | 3 | 3.8±0.6 | 2 | 3.6 |
| 30 | 3 | 4.7±1.0 | 3 | 4.8±1.2 |
| 32 | 9 | 4.0±1.0 | 5 | 4.4±1.5 |
| 34 | 10 | 4.7±1.0 | 1 | 3.5 |
| 36 | 5 | 4.0±0.4 | 3 | 5.1±2.1 |
| 38 | 3 | 4.7±1.0 | - | - |

3. Free fraction of serum cortisol related to parity

The possible relation between the free fraction of serum cortisol and parity was analysed in women with or without recorded clinical malaria during their pregnancy. The free fraction of serum cortisol was determined and analysed in nulliparae and multiparae with or without recorded clinical malaria in the same way as for total cortisol (section VI-F). The data of this investigation are summarized on Table VI-31.

Using a two-way analysis of variance model with factors

Table VI-31
Mean free fraction of serum cortisol of pregnant women with respect to parity and clinical malaria

| | CLINICAL MALARIA YES X ± SD | NO X ± SD | average of means |
|---|---|---|---|
| nulliparae | 4.8±1.0 (n=10) | 5.5±2.9 (n=15) | 5.2 |
| multiparae | 4.3±0.9 (n=18) | 4.2±1.2 (n=14) | 4.3 |
| average of means | 4.5 | 4.8 | |

parity and amenorrhea the interaction hypothesis that a possible difference between nulliparae and multiparae without recorded clinical malaria did not depend on the amenorrhea could not be rejected (p=0.27). Using a two-way analysis of variance without interaction no significant difference was found between nulliparae and multiparae (p=0.18), and again no difference was found between the corresponding amenorrhea (p=0.52).

With regard to the possible effects of parity and amenorrhea on the serum level of the free fraction of cortisol in nulliparae and multiparae with clinical malaria the two-way analysis of variance again did not reveal significance (interaction, p=0.41; parity effect, p=0.19; amenorrheal effect, p=0.18).The possible influence of parity has also been examined in a joint analysis of the data of women with or without clinical malaria. Since no relation with the duration of pregnancy could be established so far, this parameter was not considered.

The data used for a two-way analysis of variance with factors parity and clinical malaria are presented on table VI-31. The interaction hypothesis of an equal difference in

the means of the free fractions of serum cortisol with regard to the presence of clinical malaria in nulliparae and multiparae could not be rejected (p=0.40). On the other hand, an indication was found for a higher free cortisol fraction in nulliparae relative to multiparae when the data of women with or without recorded clinical malaria were summarized (p=0.06). No significant difference was found with respect to clinical malaria when the data were summarized for parity (p=0.54).

4. Free fraction of serum cortisol related to clinical malaria

To analyse the possible influence of clinical malaria on the free fraction of serum cortisol further, the data from nulliparae and multiparae were analysed separately with amenorrhea as a variable. Serum samples used for determination of free cortisol were collected as described for total cortisol (chapter VI-F3). The data are summarized on table VI-31.

A two-way analysis of variance of the data from nulliparae with factors clinical malaria and amenorrhea did not give a significant interaction between these factors (p=0.39) with respect to the free fraction of serum cortisol. On the basis of a two-way analysis of variance model without interaction an indication was found for differences between the free fractions of serum cortisol at different stages of pregnancy (p=0.10). With respect to this the high values of malarious women at 12 weeks amenorrhea may be of importance (see table VI-30). On the basis of the latter model no significance was found with respect to the presence of clinical malaria (p=0.77).

Analysis of the data of multiparae again showed no significant interaction between amenorrhea and clinical malaria (p=0.89), and no significant influence of either amenorrhea (p=0.48) or clinical malaria (p=0.39) on the free fraction of serum cortisol. A two-way analysis of variance with parity and clinical malaria as factors using summarized data without consideration of amenorrhea again exhibited no significance (table VI-31; section G-3).

This result combined with those of the previous section, suggests that the influence of parity on the free fraction of serum cortisol is stronger than that of clinical malaria during pregnancy, which is the opposite finding regarding total cortisol.

In view of the relation between total cortisol and clinical malaria during pregnancy (section F-3), a more detailed analysis was also performed with respect to the free fraction of serum cortisol including the non-malarious visits of women with clinical malaria.
The values of free fraction of serum cortisol obtained from pregnant women at a non-malaria visit preceding clinical malaria were compared to the values of women without a record of clinical malaria (table VI-32). Using a Student's

Table VI-32
Mean free fraction of cortisol from sera of a non-malaria visit preceding a clinical malaria visit compared to the values from visits in nulliparae and multiparae without recorded clinical malaria (not related to amenorrheal periods)

| | NO MALARIA RECORDED | | MALARIA POSITIVE | |
|---|---|---|---|---|
| | n: | X ± SD (%) | n: | X ± SD (%) |
| nulliparae | 10 | 4.3±0.3 | 6 | 4.4±1.0 |
| multiparae | 19 | 4.0±0.8 | 7 | 4.3±1.3 |

t-test, no significant differences were found either between the data of nulliparae ($p=0.69$) or between those of multiparae ($p=0.57$).
Results so far have not indicated that the free fraction of serum cortisol is dependent of amenorrhea (Table VI-30) or the presence of clinical malaria during pregnancy (Table VI-31).

Table VI-33
Free fraction of serum cortisol related to the parasite density index (PDI) in individual patients with clinical malaria

| parasites per $mm^3$: | PDI: | TOTAL CORTISOL (µmol/L) | FREE CORTISOL (%) |
|---|---|---|---|
| 600 | 4 | 0.66 | 3.7 |
| 600 | 4 | 1.15 | 9.1 |
| 780 | 4 | 0.88 | 4.1 |
| 900 | 5 | 0.51 | 4.6 |
| 1000 | 5 | 0.74 | 2.8 |
| 1000 | 5 | 1.05 | 3.5 |
| 1200 | 5 | 1.27 | 3.3 |
| 1400 | 5 | 1.90 | 6.2 |
| 1600 | 5 | 0.88 | 2 7 |
| 1920 | 6 | 0.87 | 4.3 |
| 2880 | 6 | 0.47 | 4.5 |
| 2880 | 6 | 1.09 | 4.5 |
| 3600 | 7 | 0.84 | 6.5 |
| 4080 | 7 | 0.85 | 4.3 |
| 4800 | 7 | 1.62 | 6.2 |
| 4800 | 7 | 1.11 | 4.4 |
| 5600 | 7 | 1.19 | 6.0 |
| 5600 | 7 | 1.13 | 3.9 |
| 6000 | 7 | 1.21 | 4.1 |
| 8160 | 8 | 2.29 | 6.9 |
| 9800 | 8 | 1.54 | 3.9 |
| 11600 | 8 | 0.90 | 4.2 |
| 22800 | 9 | 0.92 | 5.0 |
| 27240 | 10 | 1.05 | 2.9 |
| 28200 | 10 | 0.95 | 3.9 |
| 157500 | 10 | 1.40 | 14.3 |

Since increasing parasitaemia may cause increasing morbidity with a consequently increasing cortisol output, the free fraction of serum cortisol was analysed in relation to amenorrhea and the PDI (parasite density index). The data of 24 women with clinical malaria are summarized on table VI-33. The Pearson correlation between the serum fraction of free cortisol and the PDI gave a value of 0.25, and a value of 0.26 for the partial correlation with amenorrhea. The correlations were not significantly different from zero ($p=0.24$) in both instances.

It may therefore be concluded from the above described analysis and the known relation between total cortisol and clinical malaria, that if the fraction of free cortisol does not change, the concentration of free cortisol is proportionally influenced in the same way as total cortisol during pregnancy.

## H: SUMMARY AND DISCUSSION

The results of this longitudinal study group confirm the data in the literature, that nulliparae develop malaria more frequently than multiparae (chapter I). This higher prevalence of clinical malaria in nulliparae is independent of age. The low prevalence of clinical malaria of multiparae slightly decreases with increasing age and parity, with the possible restriction that the effect of age is more important than that of parity.
The malaria immunity of nulliparae apparently differs substantially from that of multiparae, and significant immunological changes are to be expected in nulliparae rather than in multiparae. A further reinforcement of malaria immunity in multiparae apparently is obtained with increasing age and parity.

In this study clinical malaria in nulliparae as well as in multiparae was observed more often during the first trimes-

ter of pregnancy, again confirming observations in other studies (Pingoud 1969; Gilles 1969; Bray 1979). The highest prevalence of clinical malaria was observed during the last weeks of the first trimester.
The frequency distribution of clinical malaria in relation to amenorrhea may either indicate that immunosuppression is only temporary, or that immunity switches to an effector system which is less sensitive to the pregnancy dependent immunosuppressive conditions. Recovery from a temporary immunosuppression was suggested by Brabin (1983). If loss of malaria immunity is related to the functional immunosuppression which prevents rejection of the foetal allograft, than a temporary immunosuppression may be less likely. Improved immunity after the first pregnancy and in multiparae may support the hypothesis of a switch to a less sensitive effector mechanism (Eling 1982b). With regard to this it is interesting to note that, contrary to cell mediated immunity, humoral immunity is believed not to be reduced during pregnancy (Loke 1978; Carter and Dresser 1983).
In contrast to prevalence of clinical malaria, parasite density during clinical malaria was not correlated with parity or amenorrhea in our study; once immunity fails to protect the pregnant woman, the proliferation of parasites proceeds in the same way.

The impact of clinical malaria on the pregnant woman was determined by measurement of the haemoglobin concentration, fever, and spleen enlargement. The malaria associated reduction of the haemoglobin levels was independent of amenorrhea and more severe in nulliparae relative to multiparae. Lowest haemoglobin concentrations were measured around 18 weeks of gestation, which is just prior to the physiological decrease of haemoglobin concentration in pregnancy due to the physiological haemodilution (Koller 1982). This decrease of haemoglobin concentration partly coincides with and follows upon a peak of clinical malaria prevalence between 12 and 18 weeks of gestation. A delay of about two weeks between the actual clinical malaria attack and the onset of anaemia has

been described by Gilles (1969). At least two processes may play an important role in the development of malaria associated anaemia. On the one hand loss of erythrocytes is due to parasitization and schizogony. On the other hand humoral immunological reactions directed against the surface of the erythrocytes either as auto-antibodies or directed against parasitic antigens associated with the surface of the erythrocytes may be involved in the destruction of the erythrocytes (Margraith 1948; Facer 1980). Such an apparently immunologically provoked haemolytic anaemia develops after peak infection, but is not correlated to its magnitude (Gilles 1969; Kortmann 1972). The longitudinal study of changes in the haemoglobin concentration during pregnancy showed decreasing values in the first part of pregnancy, being even lower in women with clinical malaria. In the second part of pregnancy the haemoglobin levels increased again, and at the same time the difference between the levels of women with or without clinical malaria decreased and became insignificant at delivery.
Results of this longitudinal study prove once more (Gilles 1969) that the heaviest stress on haemoglobin concentration is found between 16 and 26 weeks of amenorrhea after peaks of malaria prevalence have passed and when the pregnancy associated, physiological decrease of the haemoglobin level normally occuring at the end of 2nd trimester has not yet fully developed.

The spleen rate was higher in the group of women with clinical malaria compared to those without. This difference did not depend on parity or amenorrhea. The results indicated a higher probability to palpate an enlarged spleen during the first or second trimester which may be related to the higher frequency of clinical malaria in these periods, or may be due to a growing uterine mass which interferes with spleen palpation later on during pregnancy (see also chapter VII-B3 and VII-K).

Fever during pregnancy was significantly correlated to clinical malaria and independent of parity.

In summary, the data of the pregnant mother indicate that symptoms of a clinical malaria infection (anaemia, spleen enlargement, fever) are significantly related to malaria during pregnancy rather than parity. The Hb concentration is correlated to malaria as well as to parity.

Several reports describe an effect of malaria in the mother on the development of the foetus, particulary with regard to abortion, premature delivery, and reduced birth weight (Bruce-Chwatt 1952; Canon 1958; Spitz 1959; McGregor 1983; Watkinson 1983). In our longitudinal study group the possible impact of clinical malaria on the foetus was assessed by determination of birth weight, prematurity, and the condition of the newborn.

Since parity and clinical malaria are correlated (section VI-B), and both factors influence birth weight as well as prematurity birth weight was significantly higher in multiparae relative to nulliparae independent of recorded clinical malaria during pregnancy. Clinical malaria during pregnancy, however, did not significantly decrease birth weight. Prematurity was neither related to parity nor to clinical malaria, which is more or less to be expected, since prematurity was defined according to the WHO definition which is based on a certain limit for birth weight (WHO 1959). These findings are not in agreement with those of other investigators (see chapter I), but it has to be stressed again that this study was performed among a semi-immune population of pregnant women living in a holoendemic malaria area. Their immunity may be stronger as compared to areas with lower endemicity (Lawson 1967). A pregnancy associated suppression of immune responsiveness may therefore result in a more virulent, more damaging infection in women from areas with lower endemicity. Wikramasuriya (1935, 1937) worked in a study area with lower endemicity and observed more virulent infections in the mother, and a significant effect of malaria on birth weight and prematurity of the newborn.

The effector mechanism of immunity of the mother may be of importance. In general, cell mediated immunity is more

suppressed during pregnancy than humoral immunity (Loke 1978; Carter & Dresser 1983; Slapsys & Clark 1983). Therefore, the more immunity is based on cell mediated mechanisms, the greater the suppression of malaria immunity during pregnancy, and the more severe and more damaging the effect of the ensuing infection on the placental function.
Another possibility lies in the ability of the immune system to switch to a humoral effector mechanism which is less sensitive to the pregnancy dependent immunosuppressive conditions. Further research in immunology is necessary to evaluate this hypothesis.
The condition of the newborn was not correlated to clinical malaria but only with rank of birth: children born from nulliparae had a better start than newborns born from multiparae. The absence of an effect of clinical malaria during pregnancy could be considered in the light of the comparatively mild infection in these women. This could be in line with the absence of an effect of clinical malaria on birth weight and prematurity described above. The indicated better condition of babies born from nulliparae who suffered from clinical malaria cannot be explained. Other factors which stress the newborn and influence the given Apgar Score were not evaluated, such as the condition of the children small for gestational age. In addition, and possibly for the same reason, it could not be proven in this study that clinical malaria caused still birth or neonatal deaths, but in this respect the numbers were too small to be conclusive. It should be noted that others also did not find a relation between malaria and still birth (Reinhart 1978; McGregor 1983).
In summary, the results show that in spite of a higher frequency of clinical malaria in nulliparae, the morbidity of the infection in the mother (PDI, anaemia, fever, spleen rate) was independent of parity. The same holds largely true for the effects of the infection on the newborn (birth weight, prematurity, still birth, neonatal death).

The regulatory role of serum cortisol on malaria immunity

during pregnancy in the murine Plasmodium berghei model (van Zon et al., 1983), prompted the analysis in human P. falciparum infections.
Pregnant women without recorded clinical malaria in the longitudinal study group exhibited a linearily increasing serum concentration of total cortisol between 8 and 40 weeks of gestation if only one sample of each woman was taken into analysis. Analysis of a restricted number of paired samples may suggest a slightly more rapid increase in the beginning of pregnancy. Different increase rates between the 1$^{th}$ and 2$^{nd}$ trimester were also suggested by Carr et al. 1981, Demey-Ponsart et al. 1982, but none of these authors tested their limited data for linearity. The observed linearity in the overall group prompted acceptance of linearily increasing cortisol concentrations for the analysis of subgroups as well.

The situation in the rodent model is different (van Zon et al. 1983). The corticoid level in mice hardly changes untill approximately mid-pregnancy, than increases rapidly and returns to low levels towards parturition.
When the serum concentration of total cortisol is related to suppression of malaria immunity two mechanisms may be considered. First, the pregnancy dependent increase in cortisol might surpass a threshold value at a certain amenorrhea, which then suppresses the immune effector mechanism and allows proliferation of the parasite. This could explain the highest frequency of clinical malaria cases at the end of the first trimester. A decrease in frequency thereafter may be explained by a proportionally higher recovery rate (Brabin 1983). Loss of malaria immunity in relation to the serum cortisol level as well as loss of immunity at a certain amenorrhea were also observed in the murine malaria model (van Zon et al., 1982, 1983). The important difference between human clinical malaria and the experimental model is the possibility of control of an ongoing infection in the human, perhaps through a switch in the effector mechanism to avoid actual immunosuppression, whereas this is not observed in the (short) pregnancy period of the mice.

A second possibility is that women who develop clinical malaria during pregnancy have higher cortisol values throughout pregnancy. The data during and after clinical malaria support this hypothesis, but the data before the clinical malaria attack only give an indication that the difference increases towards the malaria breakthrough. A comparable observation was made in the murine model (van Zon et.al, 1982). It should be noted that differences between women with and without clinical malaria are probably blunted by the uncertainty that women without recorded clinical malaria in the study group in fact had no clinical malaria during their pregnancy. This problem is not present in the murine model.

Nulliparae without a recorded clinical malaria attack during the period of the study had a significantly higher total cortisol concentration than multiparae without a recorded clinical malaria. This difference was present throughout pregnancy and was not significantly affected by age. Although cortisol concentrations slightly decrease with increasing age, this decrease was not significant within each parity group. Thus the effect of parity on the cortisol level is more pronounced than the effect of age. An effect of parity on cortisol levels has not been described in human pregnancy or the murine model. It was observed, however, that gravida II mice that had no experience with the malaria parasite during their first pregnancy lost immunity in their second pregnancy when confronted with the parasite (van Zon et al., 1984). This suggests that the conditions during a second pregnancy are suitable for suppression of malaria immunity.

In addition, this suggests that the lower frequency of clinical malaria in multiparae is either due to a reinforced immune reactivity, or a switch in the effector mechanism towards less sensitive reactions, e.g. from a cell mediated towards a more humoral immunity during the first pregnancy. Since multiparae may develop clinical malaria infections that are comparable to those observed in nulliparae (see above), it may be assumed that the cortisol levels in some

multiparae are high enough to surpass the threshold level. A lower frequency of clinical malaria in multiparae may therefore depend on an adaptation of the immune system when it has been confronted with the problem during the first pregnancy. Other factors may be involved as well. Intercurrent diseases or deficient feeding, interfering with immune reactivity, can provoke clinical malaria (Lawson 1967), e.g. in multiparae. Multiparae may also develop clinical malaria when transmission did not take place during the critical phase of previous pregnancies, or that critical phase of previous pregnancies was under the umbrella of malaria prophylactic drugs.

Since both multiparae and nulliparae with clinical malaria exhibit higher cortisol levels when compared to their malaria negative counterparts, one can consider circumstances that provoke higher cortisol values in multiparae during pregnancy. An example may be found in genetically unrelated pregnancies, which in view of the histocompatibility antigens can be regarded as a biologically first pregnancy which might induce hormonal changes in multiparae, as in their first pregnancy. Such a phenomenon may also be responsible for the severe genuine toxicosis in multiparae (Need 1975; Koenig & Müller 1982).

Not all pregnancies of nulliparae exhibited clinical malaria, which seems in contrast with increased cortisol levels in all cases during pregnancy. The reason is not clear. In the human situation several factors may be involved, e.g. no transmission in the area, a seasonally dependent low transmission during the critical period of pregnancy, controlled or uncontrolled prophylactic antimalaria treatment, clinical malaria occurred, but was not recorded, or treated elsewhere. In all but the last two possibilities the individual may have been immunosuppressed, but the parasite was not there to provoke a clinical infection, or was inhibited on its proliferation. Such a limiting condition can be controlled in an animal experiment, and even then a proportion of primigravid mice does not loose immunity (van Zon et al., 1980a,b), for reasons that can only be speculat-

ed upon. A reasonable hypothesis is that the effector system of immunity is not identical, or not equally strong in all individuals, and therefore not equally sensitive to the immunosuppressive conditions during pregnancy.

At last, another possible explanation why not all nulliparae develop clinical malaria is the following: women, who aborted or delivered prematurily before 28 weeks of gestation in their first pregnancy, were possibly challenged to parasite proliferation and thus already switched to another effector mechanism of malaria immunity. Since no account was given to gravidity but only to parity in this study for reasons mentioned earlier (see chapter II), these women were considered as nulliparae. They did not yet give birth to a live born child but may already have developed an adapted immune response to malaria parasites.

Clinical malaria during pregnancy was correlated to significantly higher cortisol values independent of parity and amenorrhea. The significant difference in cortisol levels between nulliparae and multiparae with respect to malaria is independent of amenorrhea but may be more pronounced in the beginning of pregnancy, and gradually disappears for as yet unknown reasons.

The increase in the serum concentration of total cortisol in relation to clinical malaria gradually obscured the decrease in relation to parity during pregnancy. When clinical malaria during pregnancy is related to the serum level of total cortisol the question arises whether serum cortisol levels can have a prognostic value. When parity is not considered, the daily increase rate in the serum concentration of total cortisol was higher in women who ultimately developed clinical malaria as compared to women without recorded clinical malaria. The difference was however not significant at the 5% level. Both in nulliparae and multiparae the serum levels of total cortisol of women without recorded clinical malaria or with clinical malaria later were not significantly different two months previous to the clinical malaria visit, but the daily increase rate in relation to clinical malaria significantly increased towards the malaria visit

in nulliparae. Comparable observations were made in the murine Plasmodium berghei model (van Zon et al., 1982). The serum level of cortisol is not only significantly higher during clinical malaria but apparently remains higher even when the malaria infection is controlled. If higher serum cortisol levels were due to clinical malaria a reduction to normal levels observed in women without recorded clinical malaria would be expected after spontaneous recovery or chemotherapy. The higher cortisol values at malaria negative visits after a visit with clinical malaria in comparison to data of women without recorded clinical malaria, therefore, support the idea of higher cortisol levels in women with clinical malaria during pregnancy. It should be emphasized, however, that the actual serum level of total cortisol is not a decisive parameter for loss of malaria immunity. For example the cortisol level of nulliparae two months before a visit with clinical malaria was not significantly different from the level of nulliparae without recorded clincial malaria, but was as high as that of multiparae with actual clinical malaria (for discussion, see above).

For a more detailed description of the serum level of total cortisol of nulliparae and multiparae before and after clinical malaria, and the difference with data obtained from women without clinical malaria during pregnancy a greater number of sampling points is needed.

The serum level of total cortisol was not related to the degree of parasitaemia. On the one hand this supports the hypothesis of a causal relation between cortisol and loss of malaria immunity, rather than an increase in serum cortisol due to clinical malaria. On the other hand, however, a more detailed analysis of the clinical malaria infection in relation to cortisol values is needed, because both during a primary infection as well as during a recrudescent infection during pregnancy an increase, or even excessive increase in the serum corticoid level (particulary free corticosterone) in relation to the parasitaemia was noted in the murine malaria model (van Zon et al., 1982).

With respect to the immunosuppressive properties of serum glucocorticoids it has repeatedly been reported that free cortisol rather than the total cortisol concentration is biologically active (Slaunwhite et al., 1962).

This raises the question whether the relationship observed between clinical malaria during pregnancy and the serum concentration of total cortisol in fact also holds true for the serum concentration of free cortisol. For reasons described in section VI-G this problem was analysed through the comparison of changes in serum concentration of total cortisol with those of the free cortisol fraction. Thus, a constant free fraction of serum cortisol in combination with a linearly increasing serum concentration of total cortisol indicates an increase in the concentation of free cortisol parallel to that of total cortisol. This linear increase in serum free cortisol during pregnancy was observed in all pregnant women, regardless of parity and the presence or absence of clinical malaria. Since the serum concentration of total cortisol is higher in nulliparae relative to multiparae, being significantly higher in case of clinical malaria in both instances, and an equal free fraction of serum cortisol in nulliparae and multiparae with or without clinical malaria, a higher concentration of free cortisol is present in nulliparae relative to multiparae, again with a further increase in case of clinical malaria.

As has been mentioned before, the increase of total cortisol in case of clinical malaria was greater than the decrease relative to parity. Free cortisol concentration showed the opposite effect: a greater increase of free cortisol concentration of nulliparae compared to multiparae was measured than the increase caused by clinical malaria.

Through the same reasoning and parallel to the changes observed in the serum concentration of total cortisol, the serum concentration of free cortisol was the same when measured at a non-malaria visit previous to a visit with clinical malaria as compared to the level observed in women without recorded clinical malaria at comparable periods of amenorrhea. In addition, the free cortisol concentration was

increased during clinical malaria, but independent of the parasitaemia. In the rodent model an excessive increase in the concentration of free cortisol was observed later during an infection and without an increase of the total corticoid concentration (van Zon et al., 1983). In contrast to the human situation, in mice these infections as well as a large proportion of the recrudescent infections during pregnancy are lethal. Disturbance of the normal physiology and reduction of the production of CBG under these conditions may explain the increasing levels of free corticoid in serum of mice (Eling, et al., 1977; van Zon et al., 1978, 1983). The difference between the serum concentration of free cortisol found in women after a visit with clinical malaria in comparison to the concentration in women without recorded clinical malaria at a comparable amenorrhea could be explained along the same line.

Likewise, the mean increase rate of the serum concentration of free cortisol was higher in nulliparae with clinical malaria in comparison to those without clinical malaria, and the difference between these groups increased towards a malaria infection. In summary, the changes observed with regard to the serum concentration of total cortisol are closely followed by changes in the serum concentration of free cortisol. The parallelism between the total and free concentration of cortisol does not allow the indication of either the free or the total concentration of serum cortisol as the factor responsible for immunosuppression. However, like in the murine model (van Zon et al., 1983), the serum concentration of total cortisol is a suitable parameter.

Moreover, Rosner (1972, 1982), showed that some of the immunosuppressive effects of cortisol can be induced equally well by both free cortisol as well as the CBG-cortisol complex. In contrast to the studies in the murine pregnancy model the serum concentration of free cortisol is increased during pregnancy in women, which is in line with the observations described by Predine et al. (1979), Nolten and Rueckert (1981), Cousins (1983); see also chapter I.

Our results of a higher free cortisol concentration in nulliparae relative to multiparae have not been described previously, although Nolten and Rueckert (1981) who studied primigravidae only, noticed higher free cortisol concentrations, in comparison to other authors including also multiparae in their study. It should be pointed out, however, that our conclusions are based on indirect analysis of the free concentration and that the data in some subgroups are limited. The observations described in this chapter support a role for serum cortisol in the regulation of malaria immunity during pregnancy. The strong indications for a regulatory role of serum cortisol from the murine malaria model (van Zon 1984, thesis) are a framework and a reference for the interpretation of the data in human pregnancy. It is important to note that the selection of the proper individuals for a given analysis is not easy in the murine model, but sometimes even impossible to perform in the human situation. For example, in order to show loss of malaria immunity it is essential that the parasite is present during the period of immunosuppression. This period is not yet clearly defined. In the animal model subinoculation of blood from a pregnant mouse into a non-immune mouse can confirm the premune state until that moment, and the possibility is given of proliferation of the parasite and development of clinical malaria when pregnancy dependent immunosuppression breaks malaria immunity. Ascertainment of such a situation in human malaria may be very difficult. It is to be expected that such problems may have a blunting effect on the expected differences between groups, and it is therefore, the more encouraging that despite these problems a correlation is found between serum cortisol levels and loss of malaria immunity during pregnancy in humans.

CHAPTER VII

STUDY GROUP OF WOMEN AT DELIVERY

## A: INTRODUCTION

Fifty two pregnant women, who came to the hospital for delivery were included in this study group. Parasitaemia of the mother, the newborn, and the placenta were analysed in relation to parity, placental pathology, condition of the newborn, and the concentration of total as well as the free fraction of cortisol in the serum of the mother. The reason for the use of the data on the free fraction of serum cortisol rather than the calculated free concentration has been discussed elsewhere (chapter VI-G). In addition, the serum concentration of total cortisol and the free fraction of cortisol of the mother, and the concentration of total cortisol in the serum of the newborn were analyzed in relation to parasitaemia, parity, and mode of delivery. Serum samples taken during the first week after delivery were analyzed to determine return of total cortisol levels to non-pregnant values.
For some general considerations with regard to the problems of an analysis of the relation between serum cortisol and loss of malaria immunity during pregnancy in a clinical study the reader is referred to the introduction (p. 8).

The general characteristics of the women in this study group, e.g. age, parity, death rate (=neonatal death + stillbirth), and haemoglobin at the last antenatal clinic visit, were compared to those of other study groups (Table VII-1). No significant differences were found with the longitudinal, and the cross-sectional study group (see Table VII-1). The percentage of abnormal deliveries was compared between this study group and the total population of women who delivered in the hospital in 1982 (Table VII-1). Abnormal delivery included: breech, version and extraction, deli-

Table VII-1
Comparison of general characteristics of the different study groups of pregnant women and the total group of women who delivered in the hospital in 1982.

| Study group: | n: | AGE: X±SD | PARITY: X | HB(g%): X±SD | DEATH-RATE: | ABNORMAL DELIVERY: |
|---|---|---|---|---|---|---|
| at delivery | 52 | 26±7 | 2.6°* | 10.2±1.8 | 5.5%°* | 14.8% |
| longitudinal | 774 | 26±7 | 2.7° | 10.3±1.8 | 4.1%° | |
| cross-sectional | 242 | | 3.0* | | | |
| hospital 1982 | 709 | | | | 7.0%* | 12.5% |
| | | p 0.10 (Stud.t) | *p>0.10 °p>0.10 (Stud.t) | | *p>0.10 °p>0.10 ($X^2$-test) | p>0.10 ($X^2$-test) |

very by forceps or vacuum extraction, symphysiotomy or decapitation. Since Caesarian sections were not included in the study group, they were also excluded from the hospital group of 1982. A $X^2$-test for a 2x2 table revealed no significant difference between both groups (p>0.10).
Most women of the study group at delivery were Bantus; some were Indian or Maasai, and all originated from the study area.

B: MALARIA AT DELIVERY

1. Malaria prevalence

Malaria prevalence was determined by the presence of parasites in a thin smear from both peripheral and placental blood, and in the placental biopsy. The presence of malaria pigment in a placental biopsy was not considered to be a criterion for patent malaria, though it indicates that the woman had experienced malaria during pregnancy.

Two twin births were present in this study group; no pigment or parasites were found in either of the placentae of these twins. In the calculation of malaria prevalence each pair of placentae was considered together.

The prevalence of parasites in placenta and peripheral blood of primiparae and multiparae is summarized in Table VII-2.

Although in all women with peripheral parasitaemia parasites were also found in the placenta, the reverse was not true; only 8 out of 17 cases with a positive placenta also exhibited a peripheral parasitaemia. Thus, malaria incidence is significantly higher in the placenta ($p<0.01$; Mc.Nemar's test).

The parasite rate in both peripheral blood and placenta is significantly higher in primiparae relative to multiparae (Table VII-2). Infection of the placenta without a concomitant infection of the peripheral blood occurred significantly more frequently among multiparae (Mc Nemar' test; $p<0.01$), but not among primiparae (Mc Nemar' test; $p>0.10$).

Table VII-2
Parasite rate in placenta and peripheral blood of primiparae and multiparae

| | PRIMIPARAE: | MULTIPARAE: | $\chi^2$-test |
|---|---|---|---|
| placenta positive | 7 (58%) | 10 (25%) | $p<0.05$ |
| peripheral blood positive | 5 (42%) | 3 ( 8%) | $p<0.01$ |

With regard to the parasite rate related to parasites in the peripheral blood the data of this group can be compared to those of the cross-sectional study group (chapter V).

Analysis of the results shown in Table VII-3 indicates that the parasite rates of women at delivery, and those in the cross-sectional study group are not significantly different.

A further analysis with regard to the effect of parity also

does not reveal significant differences between these groups.

Table VII-3
Comparison of the parasite rate of peripheral blood of women at delivery and of the cross-sectional study group

| | PARASITE RATE | | |
|---|---|---|---|
| | total: | primiparae: | multiparae |
| women at delivery; n=52 | 15% | 42% | 8% |
| cross-sectional study; n=242 | 16% | 29% | 13% |
| $X^2$-test; | p>0.10 | p>0.10 | p>0.10 |

2. Parasite density

The PPDI (positive parasite density index) of placental smears was compared to the PPDI of peripheral bloodsmears of women with parasites in both smears (Table VII-4). A significantly higher PPDI was found in placental tissue as compared to peripheral blood, and this was also true when analysed for primiparae and multiparae.

Although placentae of primiparae relative to multiparae are more frequently infected (see Table VII-2), the analysis of data of all women with parasites in their placenta, independent of a concomitant infection in the peripheral blood showed that the PPDI of primiparae was not significantly different from that of multiparae (Table VII-5). The same holds true for the PPDI of the peripheral blood (Table VII-5).

The surmise arose that a positive peripheral bloodsmear was associated with a higher parasite density in the placenta. This possible association was also analysed in relation to parity. The results of this analysis (Table VII-6) indicate that the PPDI from placentae is significantly higher when the peripheral blood is also positive in primiparae, but not

Table VII-4
PPDI of placental and peripheral bloodsmears from primiparae and multiparae with parasites in both smears

| | PPDI placental smear: X ± SD | PPDI peripheral smear: X ± SD | Stud.t-test p value |
|---|---|---|---|
| total group (n=8) | 7.3±1.8 | 4.4±1.4 | p<0.001 |
| primiparae (n=5) | 7.8±1.9 | 5.0±1.2 | p=0.01 |
| multiparae (n=3) | 6.3±1.5 | 3.3±1.2 | p=0.04 |

Table VII-5
PPDI of placental and peripheral bloodsmears from primiparae and multiparae

| | PLACENTAL PPDI: X ± SD | PERIPHERAL PPDI X ± SD |
|---|---|---|
| primiparae; | 6.3±3.0 (n=7) | 5.0±1.2 (n=5) |
| multiparae; | 5.7±1.6 (n=10) | 3.3±1.2 (n=3) |
| Student's t-test: | p=0.61 | p=0.11 |

in multiparae. The difference in primiparae is apparently responsible for the significant difference when the group is analysed irrespective of parity.
The results described in this and the previous section, show a higher malaria prevalence in primiparae relative to multiparae in the peripheral blood as well as in the placenta, but the number of parasites (PDI), did not differ between both parities in blood or placenta. The parasite rate of

Table VII-6
PPDI of placental smears from women with or without peripheral parasitaemia

| | POS.PLAC.SMEAR: n: | X ± SD | POS.PLAC.+PERIPH.SMEAR: n: | X ± SD | Stud.t-test |
|---|---|---|---|---|---|
| total group | 9 | 4.6±1.9 | 8 | 7.3±1.8 | p<0.02 |
| primiparae | 2 | 2.5±0.5 | 5 | 7.8±1.9 | p<0.01 |
| multiparae | 7 | 5.4±1.6 | 3 | 6.3±1.5 | p>0.10 |

placental and peripheral bloodsmears did not differ in the group of primiparae, whereas multiparae more often showed a positive bloodsmear in the placenta than in the peripheral blood. If a primiparous woman had a peripheral parasitaemia, a more severe infection was found in her placenta than in placentae of women with placental infection only.

3. Spleen rate at delivery

The spleen rate was determined in 33 women while in the supine position and according to the classification of Hackett (see chapter II) within one hour after delivery.
One of the women, who also belonged to the longitudinal study group, exhibited an enlarged spleen after delivery, which had not been found during pregnancy, nor did she exhibit malaria parasites or malarial pigment in the placenta. This woman developed a crisis during labour, and many sickling erythrocytes were found in the placental tissue. Since in this case spleen enlargement was apparently related to sickle cell disease, this woman was excluded from the group used for analysis. The results of the examinations of 32 women are depicted in Table VII-7.
The results show that the women in this study group had a spleen rate of 34.4%. When this figure is compared to the spleen rate obtained in other study groups (Table VII-8) it appeared to be remarkably high.
Seven out of the 32 women included in this study group had

been examined earlier during their pregnancy. Four out of these 7 women exhibited an enlarged spleen (Hackett size 2) after delivery, but in only one case was an enlarged spleen diagnosed during pregnancy.
Though the number of women (7) is small, the results were in accordance with the general experience of the medical staff of the hospital.

Table VII-7
Number of enlarged spleens after delivery

| | |
|---|---|
| women without enlarged spleen: | 21 |
| women with an enlarged spleen: | 11 |
| Hackett size 1: 3 | |
| Hackett size 2: 7 | |
| Hackett size 3: 1 | |

Table VII-8
Comparison of spleen rates of several study groups

| | SPLEEN RATE: |
|---|---|
| longitudinal pregnant study group: | 2.4% |
| cross-sectional pregnant study group: | 3.3% |
| non-pregnant study group: | 9.5% |
| study group of parturient women: | 34.4% |

The measurement of the spleen size immediately after the delivery is apparently no longer hampered by the physical condition of the woman like during pregnancy, and offers a better opportunity to analyse the relation between spleen size and malaria in pregnant women.
Spleen rates were calculated in the following groups: women with a parasitaemia in the peripheral blood independent of findings in the placenta; women with a parasitized placenta,

and women with malarial pigment in the placenta. Table VII-9 shows the different spleen rates and the probability of a significant relation between spleen enlargement and either actual malaria or previous malaria.

Table VII-9
Spleen rates in the different studied groups after delivery

| different study groups | ENLARGED SPLEEN n: | % | Fisher's test: |
|---|---|---|---|
| peripheral parasitaemia | 4 | 13 | p=0.38 |
| placental parasitaemia | 7 | 23 | p=0.05 |
| malaria pigment in the placenta | 9 | 28 | p=0.02 |

Actual peripheral parasitaemia was not significantly related to spleen enlargement, though a significant relation was found when placentae were infected. This is remarkable because all women with peripheral infection exhibited placental infection as well. A significant relation was also found for an increased spleen rate, and malarial pigment in the placenta, being independent of the actual presence of parasites in the organ.
Malarial pigment in the placenta apparently indicates prolonged contact with the parasites.

C: HISTOPATHOLOGY OF PLACENTAE

The 54 placentae - 50 of single and 4 of twin-births - of the cases included in study group IV were examined histologically (for methods see chapter II).
The placentae were classified in the following groups:
1) placentae without symptoms of an active or passed malaria infection, i.e. without parasites or malarial pigment.

Some of the abnormalities seen in these placentae were correlated to obstetrical complications such as prematurity, postmaturity or prolonged labour. In addition, all four placentae of the two twin-births fell into this group.

2) placentae containing malarial pigment but lacking parasitized erythrocytes. It was assumed that these women had experienced a malaria infection earlier during pregnancy.

3) placentae containing parasites, indicating an actual malaria infection.

The frequencies of histological features in the placentae of these three groups are summarized in figure VII-1.
The frequency and the diversity of pathological changes was progressively increased in placentae with malarious symptoms or an actual malaria infection (figure VII-1).

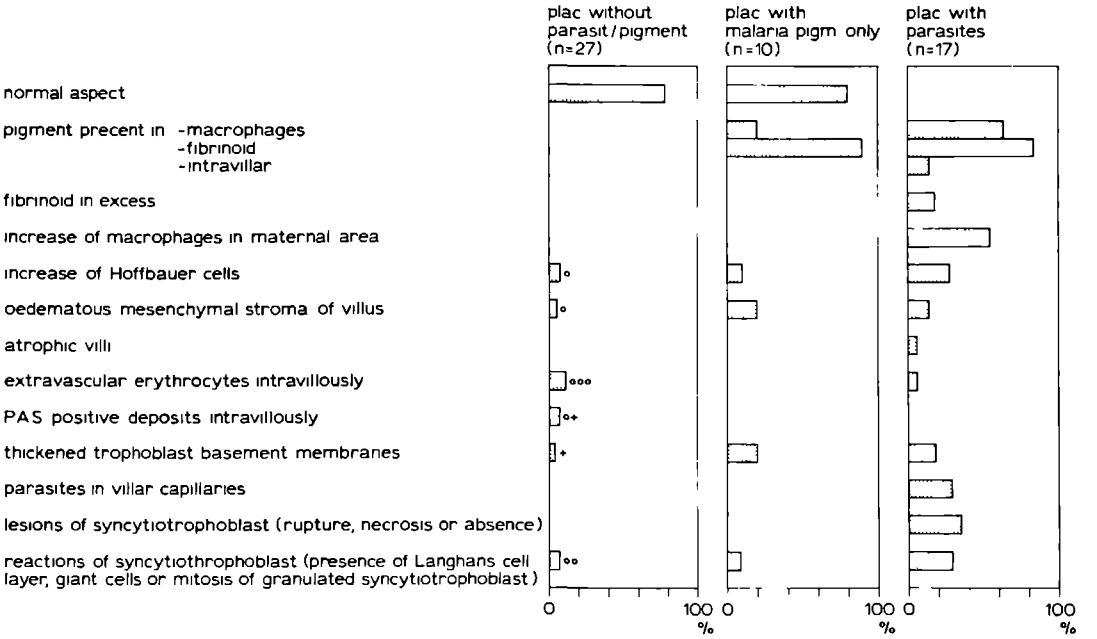



Fig.VII-1; Histological features in the placentae of women of study group III (n=52)

Histological abnormalities in placentae without malaria were only found in placentae of premature or postmature born children. No abnormalities were found in all other non-infected placentae.

Several histopathological abnormalities observed in placentae from women with malaria during pregnancy were frequently observed within the same focus.

The trophoblast basement membrane (TBM) was occasionally thickened in an irregular focal or a diffuse pattern. This change was associated with either the accumulation of fibrinoid masses, or with lesions, or with the detachment of the syncytiotrophoblast from the TBM. Large fibrinoid masses were mostly overgrown by syncytiotrophoblast, but smaller fibrinoid masses were frequently not. Small fibrinoid masses not yet covered by syncytiotrophoblast apparently develop on TBM being exposed to the intervillous space, secondary to the rupture of the syncytiotrophoblast.

Occasionally macrophages containing malaria pigment or parasitized erythrocytes were attached to the naked surface of a fibrinoid mass, whereas macrophages containing malaria pigment only were included throughout the fibrinoid mass.

Necrosis, rupture or detachment of the syncytial lining from the TBM appeared to be associated with the local presence of pigment-loaded macrophages within or between the villi.

These syncytial lesions appeared to be a "port of entry" by which parasitized erythrocytes or free parasites from the intervillous space migrated to the intravillar cappillaries (photograph 1). The lesions described here were associated with parasitaemia in the newborn (villar capillaries, figure VII-1; umbilical blood chapter VII-D).

Since the prevalence of malaria was higher in primiparae compared to multiparae, the possible effect of parity on histopathological changes in the placentae was analysed as well. The results are summarized in figure VII-2.

These results indicate that histopathological changes which are suggestive of a prolonged disturbance of placental function, such as atrophic villi, intravillar haemorrhage, thickened TBM, and abundance of intervillar leucocytes, are more frequently associated with primiparae.

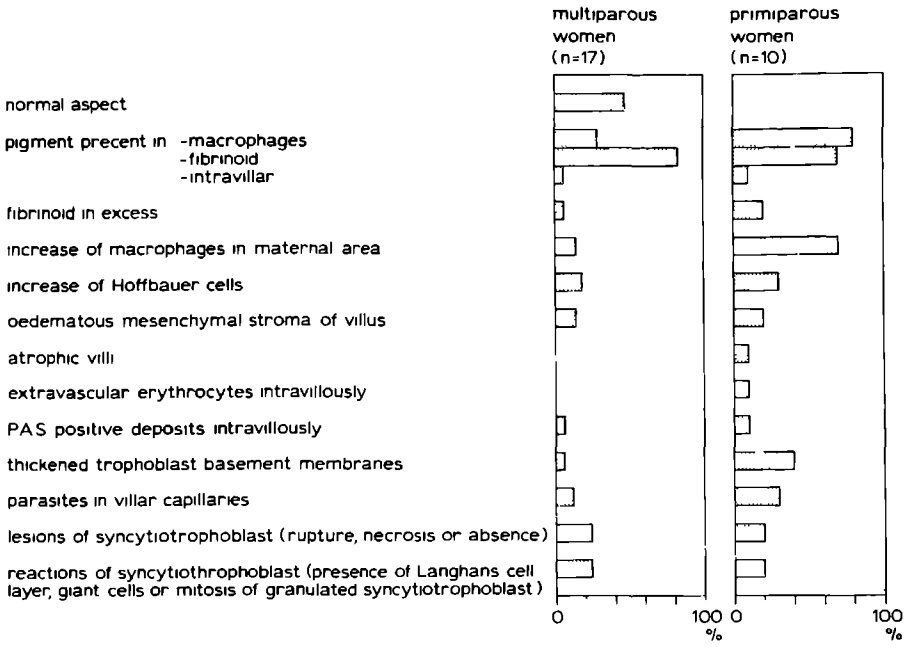
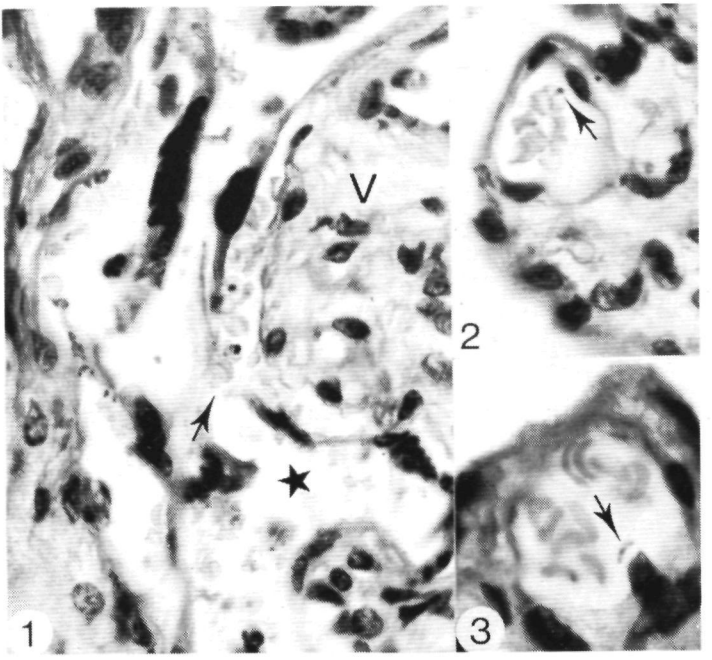



Fig.VII-2; Histological features in parasite or pigment positive placentae (n=27)

In summary it may be concluded that malaria infection, actual or passed, can cause histopathological abnormalities of the placenta. The frequency of these changes increases from non-active towards active malaria infection. In addition histopathological changes were more severe in primiparae than in multiparae. The observations therefore suggest a causal relation between a malaria infection and chronologically an increase of macrophages, lesions of the syncytiotrophoblast, exposure of TBM to maternal factors, and subsequently the formation of excess fibrinoid.
Damage of the trophoblast can be caused by activated phagocytic cells of maternal origin lying in its vicinity.
Congenital infection appears to be related directly to the lesions of the foeto-maternal barrier, and a subsequent migration of parasitized erythrocytes, and free parasites into the intravillar spaces and capillaries.




photograph 1: rupture of the syncytiotrophoblast and basement membranes (see arrow) with penetration of parasitized maternal erythocytes into the villous (see arrow); * intervillous space; V intravillous space

photograph 2 and 3: parasites within the villous (see arrow)

## D: CONGENITAL MALARIA

Parasitaemia was determined from a thin bloodsmear made from the umbilical vein. The presence of parasites in these thin bloodsmears coincided with the presence of parasites in the foetal capillaries or veins of placental tissue (photographe 2 and 3). In addition the possible effect of parity on the frequency of congenital malaria was analysed (Table VII-10). Congenital malaria as determined by infection of umbilical blood, was found significantly more frequently in babies born from primiparae than in multiparae (Table VII-10).

Table VII-10
Prevalence of congenital malaria in relation to parity

| | n: | POS. UMBILICAL BLOOD SMEAR: |
|---|---|---|
| total group; | 52 | 5 (9.6%) |
| primiparae; | 12 | 3 (25%) |
| multiparae; | 40 | 2 (5%) |

($X^2$-test; $p<0.001$)

The analysis of a possible relation between congenital malaria and the placental PPDI (Table VII-11) indicated a higher mean placental PPDI in cases with congenital malaria, but the difference compared to the group without congenital malaria is not statistically significant.

Table VII-11
Placental PPDI in relation to congenital malaria

| | PLACENTAL PPDI | |
|---|---|---|
| | n: | X ± SD |
| no congenital malaria; | 47 | 5.4±1.9 |
| congenital malaria; | 5 | 7.2±2.8 |

(Stud.t-test; p=0.14)

Table VII-12
Placental PPDI in case of congenital malaria in relation to parity

| | n: | PlACENTAL PPDI X ± SD |
|---|---|---|
| primiparae; | 3 | 9.0±1.0 |
| multiparae; | 2 | 4.5±2.1 |

Though the number of cases with congenital malaria was small (5) there appeared to be a higher placental PPDI in primiparae (Table VII-12).

Cases with umbilical parasitaemia either exhibited no clinical malaria during the first days after birth or were treated with antimalarial drugs.

## E: CHARACTERISTICS OF THE NEWBORN IN RELATION TO PLACENTAL AND PERIPHERAL MALARIA

The relation between birth weight, prematurity, condition of the newborn (Apgar score), and malaria were also analysed in the group of women after delivery as well as in the longitudinal study group. Analysis was performed with respect to peripheral as well as placental malaria. Moreover, prematurity was not determined by birth weight (see also chapter VI) but by gestational age (36 weeks or less), according to the Lubchenco score (see chapter II).

### 1. Birth weight

An influence of malaria during pregnancy on birth weight has repeatedly described in the literature (chapter I).
Birth weight is also related to parity, as is the frequency and severity of malaria during pregnancy. Therefore, the relation between malaria, parity, and birth weight was analysed in a two-way analysis of variance (complete model; see also chapter VI-D4; Table VI-18).
The twins and one child with unknown birth weight were excluded from the study group and the remaining women were classified in the subgroups primiparae/multiparae and with/without malaria. Malaria was diagnosed by a positive thin smear independent of other clinical symptoms. The mean birth weight was calculated for each class, and is presented in Table VII-13.
The difference between birth weight of children from primiparae and multiparae did not depend on malaria (interaction test: $p=0.93$).

Table VII-13
Mean birth weight (±SD) in relation to parity and peripheral parasitaemia of the mother

| | NO-MALARIA: | MALARIA: |
|---|---|---|
| primiparae; | 2625±339 g (n=7) | 2595±408 g (n=5) |
| multiparae; | 3010±542 g (n=34) | 3017±151 g (n=3) |

An almost significant lower mean was found for the birth weight of children from primiparae compared to multiparae (p=0.06); using an additive analysis of variance model (i.e. after excluding interaction), the difference was significant (p=0.03). No significant difference was found however, between the mean birth weights of children born from women with malaria relative to women without malaria (p=0.96).

Through placental investigation, infections can be recognized more precisely than in peripheral blood.
Placental infection may be of disadvantage to the development of the foetus and may be a better parameter in the birth weight analysis.Analysis of the data using placental infection as the criterium for malaria (Table VII-14a) again

Table VII-14a
Mean birth weight (±SD) in relation to parity and placental parasitaemia

| | NO-MALARIA: | MALARIA: |
|---|---|---|
| primiparae; | 2645±412 g (n=5) | 2589±334 g (n=7) |
| multiparae; | 3037±575 g (n=27) | 2938±343 g (n=10) |

showed that the difference in birth weight of children from primiparae and multiparae was not significantly dependent on placental infection (interaction test; p=0.91). The difference in birth weight between children from primiparae and multiparae was significant in both the malaria and the non-malaria group (p=0.04). Although placental parasitaemia corresponds better with a lower birth weight than peripheral parasitaemia, the observed differences between malaria and absence of malaria is not significant (p=0.65). Parity influences birth weight more than malaria in this study group.

The same analysis of variance of the data using not only patent placental infection, but also the presence of malaria pigment as the criterium for a malaria infection in the past, did not reveal a significant difference between the birth weight of children from mothers with a present or past malaria infection of the placenta (p=0.53; interaction test p=0.20). Again the parity effect was significant (p=0.02; tabel VII-14b).

Table VII-14b
Mean birth weight (±SD) in relation to parity and placental parasitaemia or malaria pigment

| | NO-MALARIA: | MALARIA: |
|---|---|---|
| primiparae; | 2525±354 g (n=3) | 2641±367 g (n=9) |
| multiparae; | 3175±617 g (n=19) | 2836±328 g (n=18) |

2. Prematurity

Gestational age of all live-born infants was assessed using the characteristics described by Lubchenco (1970; see chapter II-C). Prematurity was defined as a live-born infant with a gestational age of 36 weeks or less. The proportional

prematurities was calculated after excluding the two twin births. The influence of parity and placental malaria was analysed (Table VII-15 and VII-16). Placental malaria was defined by parasitaemia or the presence of malaria pigment. Table VII-15 shows that the probability to give birth to a premature child was not significantly related to the presence of placental malaria. Prematurity was also not significantly related to the parity of the mother. It should be noted, however, that the number of premature children in this study was very small.

Tabel VII-15
Prematurity and placental malaria

| | PREMATURITY: n: | % |
|---|---|---|
| mothers without placental malaria (n=23); | 0 | - |
| mothers with placental malaria (n=27); | 4 | 15% |

(Fisher's test; p=0.12)

Table VII-16
Prematurity and parity

| | PREMATURITY: n: | % |
|---|---|---|
| primiparae (n=12); | 1 | 8% |
| multiparae (n=38); | 3 | 8% |

3. Condition of the newborn

The condition of the newborns was expressed by an Apgar score given 1 and 5 minutes after birth. The Apgar score of the children was related to the parity of the mothers who were subdivided with regard to presence or absence of a placental

infection (Table VII-17 and 18; see also Table VI-21, 22). Twin births were excluded.
The results show more or less uniformly high Apgar scores.

Table VII-17
Mean Apgar scores at 1 and 5 minutes from children of mothers subdivided in groups with/without placental malaria

| | PARASITE NEG. | PARASITE POS. | |
|---|---|---|---|
| All mothers; | (n=33) | (n=17) | ** |
| Apgar score at 1 min. | 8.5 | 8.6 | p=0.75 |
| Apgar score at 5 min. | 9.6 | 9.8 | p=0.73 |
| Nulliparous women; | (n=5) | (n=7) | ** |
| Apgar score at 1 min. | 8.2 | 9.0 | p=0.21 |
| Apgar score at 5 min. | 9.8 | 10.0 | p=0.32 |
| Multiparous women; | (n=28) | (n=10) | ** |
| Apgar score at 1 min. | 8.5 | 8.3 | p=0.62 |
| Apgar score at 5 min. | 9.6 | 9.7 | p=0.80 |

(** Wilcoxon test).

Moreover, statistical analysis shows that Apgar scores are independent of both parity and presence of a placental infection in the mother.
In this study group there were 2 still births (4%). In one case the mother had high fever and a parasite-positive blood smear before the onset of labour. She was treated with chloroquine and fever subsided after delivery. The child died during labour. Neither the placental smear nor the peripheral bloodsmear showed parasites after delivery. Histological examination of placental tissue revealed only few pigment deposits in the fibrinoid, an increase of young granulocytes, and oedema of the villi. There is no clear indication that the death of the child was causally related to the malaria infection of the mother. The second still birth

was caused by a prolapsed cord of the first child of a twin birth.

Table VII-18
Mean Apgar scores at 1 and 5 minutes of children from mothers subdivided in primi/multiparae and groups with/without placental malaria

| | PRIMIPARAE: | MULTIPARAE: | |
|---|---|---|---|
| All children; | (n=12) | (n=38) | ** |
| Apgar score at 1 min. | 8.7 | 8.5 | p=0.92 |
| Apgar score at 5 min. | 9.9 | 9.6 | p=1.0 |
| Children of mothers with a parasite neg. placenta; | (n=5) | (n=28) | ** |
| Apgar score at 1 min. | 8.2 | 8.5 | p=0.28 |
| apgar score at 5 min. | 9.8 | 9.6 | p=0.42 |
| Children of mothers with a parasite pos. placenta; | (n=7) | (n=10) | ** |
| Apgar score at 1 min. | 9.0 | 8.3 | p=0.35 |
| Apgar score at 5 min. | 10.0 | 9.7 | p=0.47 |

(**Wilcoxon test).

F: SERUM CONCENTRATION OF TOTAL CORTISOL AT LABOUR IN RELATION TO PERIPHERAL AND PLACENTAL PARASITAEMIA, PARITY, AND TYPE OF DELIVERY

Since the study of malaria in pregnancy often has been restricted to the analysis of malaria during labour (see chapter I), and cortisol may act as an immunosuppressive agent on malaria immunity, the relation between cortisol and malaria was also analysed at labour.
Blood from the cubital vein was collected immediately after birth of the placenta and serum concentration of total cor-

tisol and the fraction of free cortisol were measured as described in chapter II.

The serum concentration of total cortisol during labour (Table VII-19) is much higher than otherwise during pregnancy (Table VI-24).
Since the total cortisol concentration is correlated to parity (chapter VI-E2), and obstetrical problems during labour cause stress to the mother, total cortisol during labour was analysed in relation to peripheral and placental malaria in the mother, parity and normal or abnormal delivery. Induction of labour by oxytocine medication, version and extraction, delivery by forceps, or vacuum extraction, symphysiotomy, and one case of sickle cell trait (section VII-B3), were all classified as abnormal deliveries.
The mean cortisol data found in the various subgroups are given in table VII-19 and 20. No abnormal deliveries were present among nulliparae. The data were analysed using a three way analysis of variance (complete model).

With respect to normal deliveries, the interaction hypothesis that the difference between the mean cortisol concentration in serum of women with or without malaria was the same for primiparae and multiparae could not be rejected neither in cases of peripheral ($p=0.77$), nor in cases of placental parasitaemia ($p=0.35$).

With respect to multiparae, again the interaction hypothesis that the difference between the mean cortisol concentration of women with or without malaria was the same for normal and abnormal deliveries could not be rejected (peripheral parasitaemia, $p=0.22$; placental parasitaemia $p=0.29$).
In addition significant differences between women with or without malaria averaged over the three subgroups was not observed neither with regard to peripheral, nor with regard to placental parasitaemia ($p=0.52$ and $p=0.55$). The influence of parity was still evident at labour, since the difference between primiparae and multiparae with respect to normal

deliveries (averaged over malaria and no-malaria) was significant for placental parasitaemia (p=0.04; according to an additive model p=0.06). In case of peripheral parasitaemia an indication was found for significance (p=0.12 in a complete model; p=0.06 according to an additive model).

Table VII-19
Mean cortisol concentration (µmol/L±SD) of groups of women classified according to peripheral parasitaemia during labour, parity and type of delivery

| | NORMAL DELIVERY | | ABNORMAL DELIVERY | |
|---|---|---|---|---|
| | no malaria | malaria | no malaria | malaria |
| primiparae | 2.2±0.5 (n=7) | 1.9±0.6 (n=5) | - (n=0) | - (n=0) |
| multiparae | 1.6±0.5 (n=28) | 1.5±0.5 (n=2) | 2.1±1.0 (n=9) | 3.0 (n=1) |

Table VII-20
Mean cortisol concentration (µmol/L±SD) of groups of women classified according to placental parasitaemia during labour, parity and type of delivery

| | NORMAL DELIVERY | | ABNORMAL DELIVERY | |
|---|---|---|---|---|
| | no malaria | malaria | no malaria | malaria |
| primiparae | 2.3±0.6 (n=5) | 1.9±0.5 (n=7) | - (n=0) | - (n=0) |
| multiparae | 1.6±0.5 (n=22) | 1.7±0.3 (n=8) | 2.1±1.0 (n=8) | 2.8±0.4 (n=2) |

Primiparous women with an abnormal delivery were not present in the study group, but abnormal deliveries in the group of multiparae averaged with respect to presence/absence of malaria exhibited significantly higher total cortisol concentrations than normal deliveries (p=0.01, peripheral; p=0.005, placental parasitaemia; Table VII-19 and 20).

## G: THE FREE FRACTION OF SERUM CORTISOL DURING LABOUR IN RELATION TO PERIPHERAL AND PLACENTAL PARASITAEMIA, PARITY, AND TYPE OF DELIVERY

The data on the free fraction of cortisol in women classified according to parity, the presence of a peripheral or placental parasitaemia during labour, and type of delivery are summarized in Table VII-21 and 22. The data were analy-

Table VII-21
Mean (±SD) fraction of free cortisol (%) of women classified according to peripheral parasitaemia during labour, parity and type of delivery

| | NORMAL DELIVERY | | ABNORMAL DELIVERY | |
|---|---|---|---|---|
| | no malaria | malaria | no malaria | malaria |
| primiparae | 10.4±3.6 (n=7) | 10.7±1.0 (n=5) | - (n=0) | - (n=0) |
| multiparae | 9.1±2.7 (n=27) | 8.2±0.0 (n=2) | 9.9±4.2 (n=7) | 9.0 (n=1) |

sed by a three-way analysis of variance (complete model) analogous to the analysis performed with respect to the total cortisol level (see chapter VII-F).
It is clear from the comparison of the data in Table VII-21

and 22 and Table VI-30, that the free fraction of cortisol is higher during labour than the pregnancy period.
With respect to normal deliveries, no significant interaction between peripheral or placental parasitaemia and parity was found (p=0.67 and p=0.85). With respect to multiparae, no significant interaction (p=0.98) between peripheral

Table VII-22
Mean (±SD) fraction of free cortisol (%) of women classified according to placental parasitaemia, parity and type of delivery

| | NORMAL DELIVERY | | ABNORMAL DELIVERY | |
|---|---|---|---|---|
| | no malaria | malaria | no malaria | malaria |
| primiparae | 11.4±3.9 (n=5) | 9.9±1.6 (n=7) | - (n=0) | - (n=0) |
| multiparae | 9.3±2.9 (n=22) | 8.2±1.3 (n=7) | 8.7±3.1 (n=6) | 13±5.7 (n=2) |

parasitaemia and the type of delivery was observed, but the difference between the free fraction of cortisol of multiparae with or without placental parasitaemia depended on the type of delivery (p=0.04).
The fraction of free cortisol did not differ significantly between women with or without malaria averaged over the three subgroups for peripheral (p=0.72) or placental parasitaemia (p=0.57). Nulliparae and multiparae both with normal deliveries, do not have significantly different free fractions of cortisol (p=0.19) in relation to peripheral parasitaemia, but the analysis regarding placental parasitaemia gave an indication for a higher free fraction of cortisol of nulliparae compared to multiparae (p=0.08).
With respect to multiparae, the free fraction of serum cortisol did not differ significantly between normal and

abnormal deliveries averaged over women with or without malaria (p=0.69 and p=0.12).

## H: THE CONCENTRATION OF TOTAL CORTISOL IN SERUM OF THE NEWBORN

Immediately after birth blood was sampled by puncture of the umbilical vene. Serum concentration of total cortisol was measured as described previously (chapter II). The effects of congenital malaria, parity of the mother and the type of delivery on the total cortisol concentration in the serum of the newborn were studied. Twins were excluded from this study group.
The data on the total cortisol concentration in serum from newborns are summarized in Table VII-23, and were analysed by a three way-analysis of variance (complete model) as in the previous section.

Table VII-23
Mean ( µmol/L±SD) serum concentration of total cortisol of newborns classified according to congenital malaria, parity and type of delivery

| | NORMAL DELIVERY | | ABNORMAL DELIVERY | |
|---|---|---|---|---|
| | no malaria | malaria | no malaria | malaria |
| newborns of primiparae; | 0.48±0.24 (n=9) | 0.23±0.50 (n=3) | - (n=0) | - (n=0) |
| newborns of multiparae; | 0.33±0.17 (n=28) | 0.13 (n=1) | 0.62±0.30 (n=7) | 1.17 (n=1) |

The interaction hypothesis that for normal deliveries the difference between mean cortisol concentrations of newborns with or without congenital malaria did not depend on parity

could not be rejected (p=0.84). This difference appeared to depend, however, on the type of delivery (interaction test; p=0.02).

No significant difference was found between newborns with and without congenital malaria averaged over the three subgroups (p=0.77). In addition no significant difference was found between the mean concentration of total cortisol of newborns from nulliparae and multiparae with a normal delivery (p=0.32). In multiparae the children born by abnormal delivery had a significantly higher cortisol level than normally delivered children independent of presence or absence of congenital malaria (p=0.001).

## I: SERUM CONCENTRATION OF TOTAL CORTISOL AT DELIVERY AND DURING THE POST-PARTUM PERIOD

Changes in the serum concentration of total cortisol during labour and the post-partum period were analysed in primiparae with a normal delivery, and multiparae with either a normal or an abnormal delivery.
Since malaria did not cause significant difference between these study groups (chapter VII-F and G), it was not considered in this analysis. All women were sampled during labour, arbitrarily called time zero. Most women were sampled twice more during the first week post-partum. These samples were taken between 11 am and 1 pm to avoid effects caused by diurnal changes.
Since sampling times varied from 10 to 119 hours post-partum, two groups were formed: group A from 10 to 36 hours, group B from 37 to 84 hours post-partum. Samples taken later than 84 hours post-partum were not included in the analysis due to small numbers. When a woman had been sampled twice within one interval of time, only the first sample was used.

The concentration of total cortisol was determined in the sera of the above mentioned subgroups (primiparae with

normal delivery; multiparae with normal or abnormal delivery) during labour, and in the two interval periods post-partum. The means are depicted in Table VII-24a, b and c. Comparison of the mean values of these three subgroups at time 0 (labour), using an one-way analysis of variance, revealed a significant difference (p=0.01: Table VII-24a).

Table VII-24a
Mean serum concentrations of total cortisol (µmol/L) during labour of nulliparae and multiparae with a normal delivery, and multiparae with an abnormal delivery

| study groups | n: | mean: | SD |
|---|---|---|---|
| A: nulliparae/normal delivery | 12 | 2.05 | 0.52 |
| B: multiparae/normal delivery | 30 | 1.61 | 0.47 |
| C: multiparae/abnormal delivery | 10 | 2.23 | 0.97 |

"multiple comparison method":
A-B=0.44 p=0.11
B-C=0.62 p=0.03

Table VII-24b
Mean serum concentrations of total cortisol (µmol/L) in the period 10 to 36 hours post-partum of nulliparae and multiparae with a normal delivery and multiparae with an abnormal delivery

| study groups | n: | MEAN: | SD |
|---|---|---|---|
| A: nulliparae/normal delivery | 9 | 0.97 | 0.41 |
| B: multiparae/normal delivery | 28 | 0.76 | 0.23 |
| C: multiparae/abnormal delivery | 10 | 0.87 | 0.33 |

"multiple comparison method";
A-B=0.21 p=0.20
B-C=0.11 p=0.60

Using the "multiple comparison" method of Scheffé, significant differences were mainly due to the significantly higher cortisol values in multiparae with an abnormal delivery (p=0.03; Table VII-24a). The Scheffé analysis did not show a significant difference between nulliparae and multiparae with normal deliveries (p=0.11; Table VII-24a).
These results confirm those described in chapter VII-F; the p-values and the consistently higher cortisol concentrations of nulliparae indicate that nulliparae may have higher total cortisol concentrations but the influence of type of delivery is even more pronounced.

With regard to both time intervals post-partum (10 to 36 hours, and 37 to 84 hours) the one-way analysis of variance did not show significant differences between the three subgroups (resp. p=0.17 and p=0.88; Table VII-24b and 24c).

Table VII-24c
Mean serum concentrations of total cortisol (µmol/L) in the period 37 to 84 hours post-partum of nulliparae and multiparae with a normal delivery and multiparae with an abnormal delivery

| study groups | n: | MEAN: | SD |
|---|---|---|---|
| A: nulliparae/normal delivery | 6 | 0.59 | 0.12 |
| B: multiparae/normal delivery | 10 | 0.58 | 0.13 |
| C: multiparae/abnormal delivery | 7 | 0.62 | 0.26 |

"multiple comparison method";
A-B=0.07 p=1.0
B-C=0.04 p=0.89

In the interval period of 10 to 36 hours post-partum the difference between the serum concentration of total cortisol in nulliparae and multiparae with normal deliveries was about twice as big as that between multiparae with a normal

or abnormal delivery. Although these differences were not significant according to the Scheffé analysis, comparison with the differences at time zero shows that the higher total cortisol is related to the stress of an abnormal delivery and decreases more rapidly than the higher total cortisol related to parity.

When the cortisol levels from women who were sampled during labour, and twice thereafter were analysed with respect to time after delivery a significant decrease was noted. The average decrease between delivery and the period 10 to 36 hours after delivery was 1.03 µmol/L (p=0.0001), and in the period 37 to 84 hours it was 0.23 µmol/L (p=0.0005).

The individual changes in the serum concentration of total cortisol after delivery in primiparae with a normal delivery, and multiparae with a normal or abnormal delivery are depicted in figures VII-3, 4 and 5 respectively. In this analysis only the data from women sampled at least three times were included.

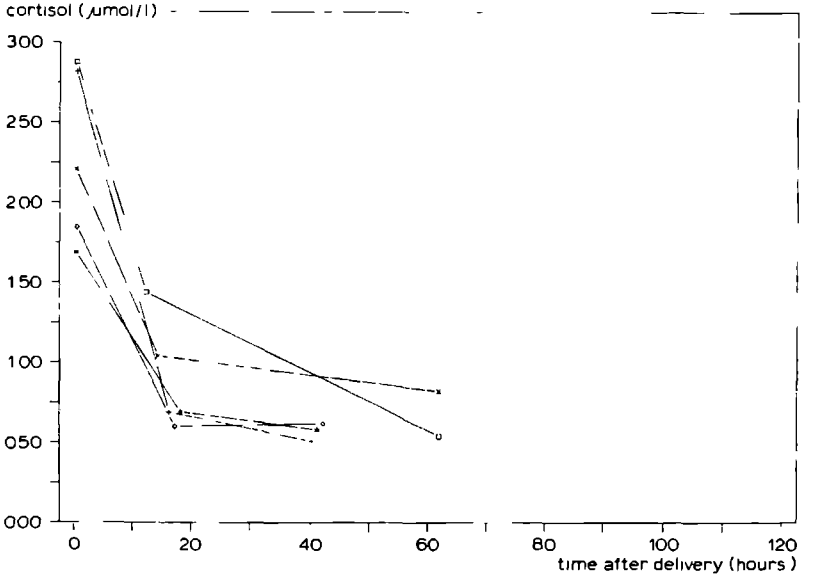



Fig.VII-3; Individual changes in the serum concentration of total cortisol of primiparous women (n=5) during (zero time) and after normal delivery

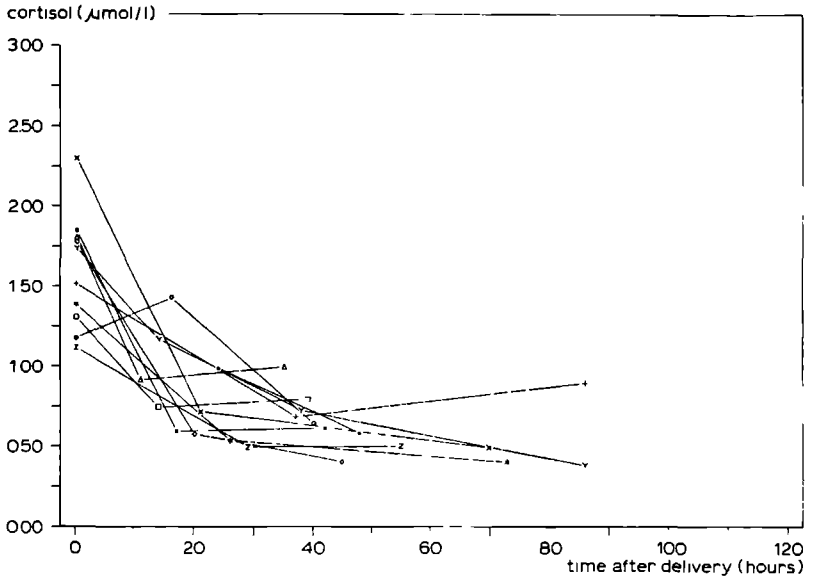



Fig.VII-4; Individual changes in the serum concentration of total cortisol of multiparous women (n=11) during (zero time) and after normal delivery

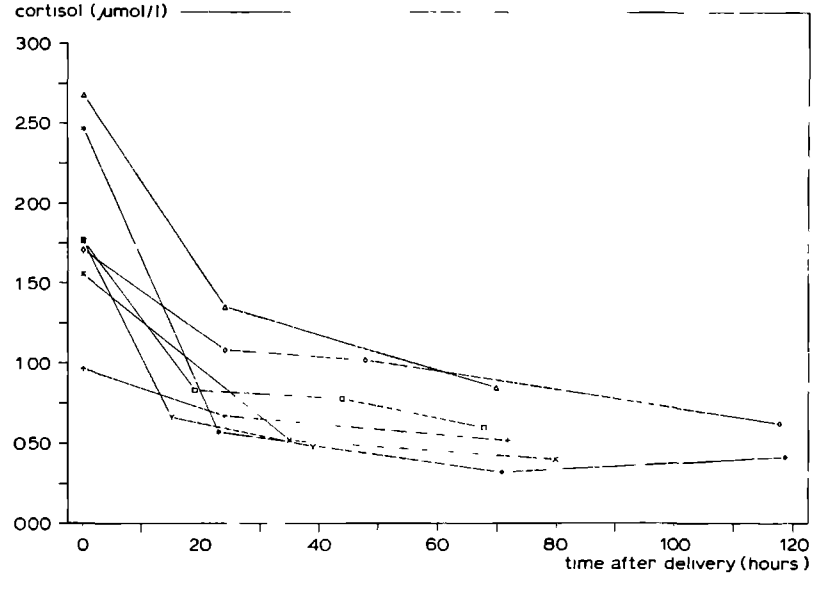



Fig.VII-5; Individual changes in the serum concentration of total cortisol of multiparous women (n=7) during (zero time) and after complicated delivery

The composition of the post-partum group, as well as some of the characteristics of the women in this group, were comparable to those of other study groups, and was analysed especially in relation to the longitudinal study group.
Postpartum analysis has been used for the study of the relation between malaria and the outcome of pregnancy (a.o. McGregor 1983, 1984). In those studies presence or absence of peripheral and/or placental infection was an important criterium for the identification of women with malaria during pregnancy. In such an approach it was assumed that the post-partum analysis still reflects the pregnancy dependent suppression of malaria immunity. On the other hand, it has repeatedly been observed that the highest frequency of malaria cases is not found post-partum, but earlier during pregnancy (12 to 18 weeks of pregnancy, chapter VI-B2 of this thesis). Brabin (1983), even claims that later during pregnancy the recovery rate surpasses the rate of recrudescence. For these reasons it is important to analyse the relation between malaria and pregnancy also in a post-partum group.
The results show that the parasite rates determined from peripheral blood are the same in the delivery group and the cross-sectional group analysed during pregnancy. A higher prevalence of malaria in nulliparae compared to multiparae was found during pregnancy and also during delivery. It was surprising to observe a significantly higher parasite rate using placental data compared to data from peripheral blood in multiparae, but not in nulliparae. The explanation for this parity dependent effect is unknown. McGregor (1984) suggested that local suppression of malaria immunity may explain the finding of a higher frequency of placental infections compared to that of the peripheral blood; sometimes a placental infection is found in the absence of parasites in the peripheral blood.
Determination of local compared to systemic immune reactivi-

ty using mixed lymphocyte reactivity (Clark et al., 1980, 1981), supports such a possibility, but leaves unexplained why the phenomenon is parity dependent.
The absence of a significant difference between the Positive Parasite Density Index (PPDI) of primiparae relative to multiparae with respect to both placental and peripheral infection suggests that the infections following loss of immunity are comparable. However, the significantly higher PPDI in women, especially in primiparae with infected placental and peripheral blood compared to women with positive placental blood only, may indicate quantitative differences in immunosuppression. More information is needed, however, before a complete and satisfying interpretation can be given.
The spleen rate as an indicator of malaria is an unreliable parameter in pregnant women. The spleen rate was much higher after delivery than during pregnancy, indicating interference of the growing uterus with spleen palpation. The spleen rate after delivery corresponded well with the frequency of histologically observed deposits of malaria pigment and/or presence of parasites in the placenta. The absence of a correlation between spleen rate and infection of the peripheral blood after delivery only seems to indicate that placental infections alone also give rise to splenic enlargement, which makes placental infection a less local phenomenon.

Histopathological analysis showed that lesions observed in relation to malaria were observed repeatedly. Damage of the foeto-maternal barrier, such as lesions of the syncitial lining, possibly acting as a "port of entry" for the parasites to the foetal circulation may be an important histopathological feature. Interaction of macrophages that may or may not contain malaria pigment with the syncitial lining and fibrinoid masses plugging syncitial lesions points in the direction of a role of macrophages in the pathogenesis of the lesions. Such an interaction was suggested in the pathogenesis of endomyocardial lesions

developing in relation to malaria (Eling et al., 1983, 1984).
In both situations the starting point may be damage of the endothelial lining mediated by a parasite dependent process, possibly involving macrophage activity.
The correlation between damage of the foeto-maternal barrier, and congenital malaria as diagnosed by a positive bloodsmear from umbilical cord blood, underlines the importance of this histopathological change in the placenta. Moreover, the significantly higher frequency of congenital malaria in primiparae compared to multiparae, and a significantly higher PPDI in primiparae with a placental infection are in line with the higher frequency of histopathological changes in primiparae compared to multiparae. Parasitaemia and also the magnitude of the infection seem to play a role in the pathogenesis of lesions in the foeto-maternal barrier, which is in accordance with the findings of Galbraith et al. (1980).

Neither birth weight nor prematurity were significantly related to patent malaria in the mother. This is not surprising in view of comparable results obtained in the longitudinal study group. In addition, it may be expected that parasitaemia during pregnancy is more likely to have an effect on birth weight than patent malaria at labour. This raises the question whether in the investigated population the morbidity of the infections was less than in other reports e.g. study groups in Nigeria which exhibited reduced birth weights in relation to malaria (chapter I; Table I-3). One factor of importance is that in our study group malaria was defined by a positive smear and not by clinical symptoms, a method which may obscure the data. Moreover, the influence of parity on birth weight was accounted for in this analysis. The significantly higher birth weight of children from multiparae compared to primiparae indicates that apparently the parity effect is stronger than the effect of patent malaria. Neither prematurity nor the condition of the child as determined by the Apgar score

appear to be affected by patent malaria in the mother.
In summary, these observations indicate that patent malaria in the mother may lead to congenital infection without affecting maturity, birth weight, or the physical condition of the child.
The serum concentration of both total cortisol and the fraction of free cortisol are strongly increased at labour compared to levels during pregnancy.
When labour is regarded as a stress condition, the increased levels of bound and free cortisol are the result of an increased ACTH release by the pituitary gland (Kauppila et al., 1974; Carr et al., 1981), despite already elevated levels of cortisol during pregnancy compared to non-pregnant individuals. The cortisol increase during normal labour, which was observed in primiparae and in multiparae, supports the hypothesis of Nolten and Rueckert (1981) who suggested that the hypothalamo-pituitary feedback mechanism has been reset at a higher level during pregnancy and can react normally to stress.
Whereas malaria during pregnancy was associated with increased levels of total and free cortisol in the serum, this phenomenon was not observed during delivery. It should be noted, however, that levels during and immediately after delivery are substantially increased, when compared to those during pregnancy. This probably stress related increase, due to delivery, may obscure the association between serum cortisol levels and malaria. On the other hand the difference in cortisol levels between nulliparae and multiparae during pregnancy is still observed during labour despite the further increase.
In view of the high cortisol concentrations during and immediately after delivery the question arises whether they may cause loss of immunity and are in fact related to malaria during and immediately after delivery. This question is complicated by the fact that levels return to normal levels shortly after delivery.
Therefore, not only the magnitude of the increase, but also the duration of this increase may be of importance with respect to suppression of malaria immunity.

Comparison with the murine model is hindered by the fact that, in contrast to the human situation, the pregnancy period is very short in comparison to the duration of an infection. Recovery observed in the human situation (Brabin 1983) is not obtained easily in the animal situation (van Zon et al., 1983), where increase in serum corticoid concentrations progresses rapidly during a recrudescence and may suppress the adjustment of immune reactivity necessary for recovery.
Complicated delivery - in this study only observed in multiparae - was associated with higher serum concentrations of total cortisol, compared to levels found immediately after normal delivery. Since the free fraction of serum cortisol remained the same, the concentration of free cortisol increased also. A complicated delivery is considered to add additional stress to that caused by a normal delivery.

The increased serum concentration of total cortisol and free cortisol in multiparae with an abnormal delivery, not dependent on patent placental infection may particulary relate to the acuteness of the cortisol release signal during delivery, which is not anticipated by an increased production of cortisol-binding-globulin. The rapid return of both cortisol levels to non-pregnant levels after delivery underlines the time limitation of the signals. Additional amounts of free cortisol produced by short term signals are rapidly excreted by the kidneys (Lindholm 1973). Longstanding increased levels are therefore expected to be associated with increased concentrations of CBG and prolonged production signals.

Congenital malaria was not reflected by changes in total cortisol in the newborn, a result which parallels the independence of total cortisol of malaria in the mother after delivery. Significantly increased cortisol levels when mothers experienced an abnormal delivery were also found in the newborn, suggesting that there is an exchange between the blood compartments of mother and child. Complicated deliveries are associated with foetal distress. Stress

reactions have been observed in the unborn child (Puolakka et al., 1981), and stimulation of the foetal sympathico-adrenal system may cause increased cortisol levels in the foetus (Murphy et al., 1975; Puolakka et al., 1983).
Transfer of cortisol from mother to foetus across the placental barrier has also been described, but such secretion only takes place if a tremendous increase of maternal cortisol concentration occurs, since most of the cortisol is transformed to corticosteron in the placenta (Murphy 1973; Murphy et al., 1974). Predine et al. (1979) described an increase in the fraction of free cortisol in cord blood during delivery and suggested that free cortisol in maternal and cord blood are either similarly regulated or that there is partial communication between both compartments.

From these observations it is likely that stress dependent changes in the serum levels of cortisol, particularly the levels of free cortisol, can be substantial and can interfere with the relation between cortisol and malaria. Changes in the serum concentration of total, rather than free cortisol, may be a better parameter for the determination of loss of malaria immunity in relation to serum cortisol. A comparable situation is found in the murine P. berghei model (van Zon et al., 1983).
It remains to be determined whether the impressive changes in serum cortisol during labour and delivery cause loss of malaria immunity. Important questions are, whether the duration of the signal is sufficient and also to what extent recovery reactions during pregnancy (Brabin 1983) make the immune reactivity of the effector system less sensitive to cortisol.

CHAPTER VIII

# GENERAL CONSIDERATIONS

Conception and the subsequent nidation of the fertilized ovum trigger many hormonal changes. These hormonal changes are involved in the protection of the foetal allograft by limiting the invasive growth of the trophoblast tissue on the one hand, and in the suppression of immunological transplantation reactions which may reject the foetal implant on the other hand (Gauchi 1981).
This suppression of immunological reactivity probably takes place on a local level in the genital tract (Slapsys 1983), e.g. placenta (uterus) as well as generalized, e.g. in the peripheral blood (Loke 1978).
The consequence of this immunosuppression is a more sensitive state of the pregnant woman to primo infections, and loss of established specific immune reactions, especially cell mediated immune responses, which may lead to recurrent disease.

Malaria is a good example for the study of loss of immunity during pregnancy in the murine model (van Zon 1982), as well as in the human situation. Premunity i.e. persistence of a subpatent number of parasites in an otherwise immune host is the type of immunity observed in both men and mice. Persistent parasites in immune hosts can provoke a recrudescence when immunosuppressive conditions break malaria immunity. Recrudescent infections, therefore, serve as an efficient marker of immunosuppression during pregnancy. Moreover, cell mediated immunity is an important factor in both, loss of immunity during pregnancy and malaria immunity (Jayawerdena 1981).
Loss of malaria immunity in women can be studied in an endemic malaria area like Turiani area (chapter III), if malariometric information concerning endemicity have been obtained from subgroups like children (chapter III), and non-pregnant fertile women (chapter IV).

Suppression of peripheral malaria immunity causes a higher parasite rate (chapter V) and clinical malaria (chapter VI), and suppression of the immune responses in the placenta causes a higher parasite rate and parasite density locally (chapter VII).

A generalized breakdown of malaria immunity is hazardous to the mother due to attacks of fever, spleen enlargement, and anaemia (chapter VI). The sequelae of the depressed immune reactivity in the placenta are reflected by the histopathological features found in the placental tissue which interfere with placental function: a slight but not significant reduction of birth weight was found in this study. Moreover, these placental lesions were the port of entry for malaria parasites in case of congenital parasitaemia (chapter VII).

Several serum factors related to the pregnant state may have immunosuppressive properties in vitro (chapter I), but only the immunosuppressive action of cortisol on the cellular immune response in vitro and in vivo has been described in detail (chapter I).

Cortisol is known to suppress T-cell dependent immune reactions and causes an inversion of the B:T cell ratio, as observed during the first trimester of pregnancy (Strelkauskas et al., 1975, 1978). Studies on the cortisol concentration during pregnancy showed a considerable increase with progressing amenorrhea (chapter VI). Moreover, this cortisol increase was parity dependent - higher in nulliparae than in multiparae - and independent of age (chapter VI). A comparable relation was obtained with respect to loss of malaria immunity in relation to parity and age (chapter VI).

Total cortisol as well as the concentration of free cortisol appeared to be higher in women with loss of their malaria immunity during pregnancy (chapter VI). Total cortisol concentration in serum appears to be a good parameter for the measurement of loss of immunity during pregnancy, as concluded from the murine malaria model (van Zon 1984). The action of cortisol on the effector response of malaria immunity apparently is not merely related to the unbound fraction of

cortisol as was already suggested by Rosner (1972), who proved that the immunosuppressive effect of free cortisol is comparable to that of bound cortisol (CBG-cortisol complex). The relation between loss of immunity and the amenorrheal period (van Zon et al., 1980a,b; Brabin 1983) as well as the substantial difference between malaria immunity of nulliparae relative to multiparae suggest an adaptation or reinforcement of the immune system. The role of an increased cortisol concentration may not only be suppression of cellular immune reactivity to prevent destruction of the foetal implant but may also create the possibility for the maternal immune system to reinforce or to induce other immune reactions, e.g. humoral responses. Altered immune reactivity may protect in a subsequent pregnancy, like in the murine malaria model (van Zon 1984).

The similarity between the murine and the human malaria model demonstrates that immunological processes in human pregnancy can be studied in the experimental murine model. Altered immune reactivity during pregnancy offers the possibility to study specific effector mechanisms involved in the defence against viral and parasitic diseases as well as certain tumor antigens.
In addition, more information about the altered immune system during pregnancy can be obtained by analysis of the defence mechanisms against other pathogens, which show a breakdown during pregnancy and from which the immune responses outside pregnancy are known.

CHAPTER I reviews the literature on the relation between malaria and pregnancy, and the effects of malaria on mother and child during pregnancy, the changes in immunity in general and especially the changes in malaria immunity, as well as the factors responsible for these changes during pregnancy.
Malaria occurs more often during pregnancy than before or after pregnancy, and a negative correlation is found with increasing parity as well as probably with increasing age. There are indications that the frequency of malaria is not increased during the whole period of pregnancy but especially at the end of the first and the beginning of the second trimester. Malaria does not occur more often during the puerperium. The prevalence of congenital parasitaemia differs in each study and probably depends on the immune status of the mother.

Malaria attacks cause anaemia of the pregnant woman. An increase of the number of abortions or premature deliveries due to malaria was not recorded by all investigators. Recorded differences as to premature deliveries are also influenced by the method used for the assessment of prematurity. Children of mothers who suffered from malaria during pregnancy generally have a lower birth weight although the observed differences are not always significant. The simultaneous effect of parity on malaria prevalence and on birth weight was frequently not analysed.

To maintain a pregnancy as a so called "foetal-allograft", adaptation of the immune system of the pregnant woman is a prerequisite. Humoral immune reactions appear to remain intact, or are even improved, whereas cell-mediated immunity is suppressed.

Those aspects of malaria immunity which are mainly T-cell

dependent, are discussed. Literature concerning depressed malaria immunity during pregnancy is described
Immunosuppressive action is ascribed to several protein fractions and hormones in serum of pregnant women. The immunosuppressive action of corticosteroids is discussed briefly, followed by a description of studies concerning total cortisol, free cortisol, ACTH, and CBG during oestrogen medication, pregnancy, labour and puerperium.

Extra attention is paid to the possible regulatory function of corticosteroids on malaria immunity in the murine-malaria model, since those results were the starting point for the study described in this thesis.

CHAPTER II describes the study area and the composition of different study groups. A short consideration on terminology used in this thesis is given. The method of measurement of the different parameters during the field research in Tanzania as well as at the laboratory for Chemical and Experimental Endocrinology (head; prof. dr. Th. J. Benraad) of the St. Radboud Hospital, Nijmegen afterwards, is discussed.
Laboratory methods used for the measurement of total cortisol in serum are discussed in detail. Special attention is given to the interassay of variance because of the many assays which were needed to measure such a large number of samples. Attention is given to the possible influence of a longlasting anti-malaria prophylaxis on loss of immunity.

CHAPTER III shows the results of two malaria surveys in which the frequency of a palpable spleen and the prevalence of parasites in the peripheral blood, pointed to a hyperendemic malaria area. Seasonal variations were analysed by means of malariometric and climatological data.

CHAPTER IV describes the frequency of both parasitaemia and palpable spleen in a reference group of healthy non-pregnant women. The concentration of total cortisol, and the fraction

of free cortisol were measured in the serum of these women. An indication was found for a slight decrease of the concentration of total cortisol with increasing age. Although the concentration of total cortisol of parasite positive women did not differ from that in parasite negative women, no further conclusions could be drawn due to the small number of parasite positive women. The same holds true for the observed higher fraction of free cortisol in case of women with parasitaemia.

CHAPTER V describes the results of the cross-sectional study of pregnant women, in which not the prevalence of clinical malaria but that of parasitaemia was studied. Parasitaemia was found more often in nulliparae than multiparae; moreover, the frequency decreased with increasing parity within the group of multiparae. No clear correlation was discovered between parasitaemia and the duration of amenorrhea. The prevalence of an enlarged spleen correlated well with parasitaemia. If fever was considered as the parameter, this correlation was not found any more. This finding was to be expected since clinical malaria (parasitaemia plus symptoms) was not analysed but only the presence of parasites. The concentration of total cortisol appeared to be higher in nulliparae compared to multiparae; the same holds true for parasite positive women when compared to parasite negative women. The measured differences were not significant during all amenorrheal periods. No relation was found between the grade of the parasitaemia and the cortisol concentrations.

CHAPTER VI reports the results of the longitudinal study of pregnant women. In this study the prevalence of clinical malaria and not only the presence of parasites in the peripheral blood was used as a marker of loss of malaria immunity. Clinical malaria was found more often in nulliparae than multiparae; age did not play a role in the nulliparae, but a slight decrease with increasing age was seen in the group of multiparae. The frequency of clinical malaria visits depended on the duration of amenorrhea: clinical

malaria occurred more frequently between 12 to 18 weeks of amenorrhea. The parasite density in case of clinical malaria was not dependent on parity or amenorrhea, but the prevalence of an enlarged spleen or fever was associated with clinical malaria.

The diagnosis of an enlarged spleen was negatively affected by an increasing amenorrhea. Haemoglobin values (Hb) during pregnancy depended on amenorrhea, parity and clinical malaria. Nulliparae and/or women with clinical malaria had the lowest Hb concentrations; in addition lowest Hb concentrations were found between 16 and 26 weeks of amenorrhea. The birth weight of children was higher in multiparae than primiparae. Clinical malaria during pregnancy only had a slight and insignificant influence on birth weight. Birth weight was used to determine prematurity of a child in this study. The frequency of prematurity was neither related to parity nor to clinical malaria. The same findings emerged from the analysis of the Apgar scores.

The serum concentration of total cortisol significantly increased during pregnancy, with a significant difference between nulliparae and multiparae. Nulliparae had higher concentrations of total cortisol independent of their age or amenorrhea. Women with clinical malaria had higher cortisol levels during and after the visit with clinical malaria in comparison to women without clinical malaria, and the difference between nulliparae and multiparae remained.

Indications were found that women with clinical malaria already had higher cortisol levels before the recrudescence. The concentration of free cortisol, derived from the fraction of free cortisol, revealed the same changes as observed for the concentration of total cortisol, though the effect of parity was more pronounced than the effect of clinical malaria.

CHAPTER VII contains the data of the study during and after labour. Parasitaemia was determined in peripheral blood of the mother, placental blood, and in blood of the umbilical vein.

A higher parasite density was found in the placenta than in the peripheral blood; the higher parasite density of primiparae compared to multiparae was more often accompanied with congenital parasitaemia. The size of the spleen was determined after delivery and appeared to be enlarged more often than expected from data obtained during pregnancy. Comparison of these findings with those from other study groups showed that examination of the spleen is an unreliable method for the measurement of malaria prevalence during pregnancy.

Histological changes in the placenta in relation to malaria strongly suggest passage of parasites and hence congenital parasitaemia.

Children of mothers with malaria infection of the placenta had a lower birth weight, but the difference was not significant. Parity, however, had a significant effect again. In this study no indication was found that women with malaria more often give birth to a premature child. Moreover, the condition of the child was not affected by a malaria infection of the mother.

Labour was associated with very high cortisol concentrations, but the parity dependent difference between primiparae and multiparae was preserved. A complicated delivery caused additional stress of the mother leading to higher cortisol concentrations. Parasitaemia of the mother or in the placenta did not affect the cortisol values of the mother or the child. Values of total cortisol of the child were also not higher in case of a primiparous mother, notwithstanding the higher cortisol levels of these mothers. The additional stress of an abnormal delivery was reflected in the neonate which could be due to an increased transfer of free cortisol through the placenta or to a stimulation of the hypophysis-adrenal axis of the child.

Concentrations of total cortisol became normal within 3 to 4 days post partum with a comparatively more rapid decrease in women with a complicated delivery, which could point to a higher concentration of free cortisol during labour in these mothers.

HOOFDSTUK I geeft een literatuuroverzicht van de samenhang tussen malaria en zwangerschap, de gevolgen van malaria tijdens zwangerschap voor moeder en kind, de veranderde immuniteit tijdens zwangerschap in het algemeen en van malaria immuniteit in het bijzonder en de daarvoor verantwoordelijke factoren.
Malaria komt vaker voor tijdens zwangerschap dan daarbuiten, waarbij een negatieve correlatie bestaat met stijgende pariteit en mogelijk ook met de leeftijd. Er zijn aanwijzingen dat een verhoogde malaria frequentie niet gedurende de gehele duur van de zwangerschap gevonden wordt, maar vooral op het einde van het eerste en begin tweede trimester. Malaria komt niet vaker voor in het kraambed. De frequentie van congenitale parasitaemie verschilt per studie en hangt waarschijnlijk af van de immunologische toestand van de vrouw. Malaria aanvallen leiden tot bloedarmoede bij de zwangere vrouw. Een toename van het aantal abortus of vroeggeboorten als gevolg van malaria wordt niet door elke onderzoeker gevonden. De gevonden verschillen ten aanzien van vroeggeboorten worden mede bepaald door de wijze waarop prematuriteit wordt gedefinieerd. Kinderen van moeders met malaria tijdens de zwangerschap hebben over het algemeen een lager geboortegewicht alhoewel de gevonden verschillen niet altijd significant zijn. Meestal werd de gelijktijdige invloed van pariteit op zowel het voorkomen van malaria alswel het geboortegewicht niet geanalyseerd.
Een veranderd afweersyteem bij de zwangere vrouw is een vereiste om een zwangerschap te laten voortbestaan als een zogenaamde "foetal allograft". Immuunreacties die voornamelijk humoraal verlopen blijken intact te blijven of zelfs beter te verlopen, terwijl de celgebonden afweer onderdrukt wordt. De kenmerken van de malaria immuniteit die voornamelijk T-cel gebonden is, worden besproken. De literatuur over verlaagde malaria immuniteit tijdens zwangerschap wordt aan een beschouwing onderworpen.

Aan verschillende eiwitfracties of hormonen in het serum van zwangeren wordt een immunosuppressieve werking toegeschreven. De immunosuppressieve werking van de corticosteroiden wordt beknopt behandeld, waarna de studies betreffende het totale en vrije cortisol gehalte, ACTH en CBG tijdens oestrogeen-medicatie, zwangerschap, partus en kraambed worden besproken.
De mogelijk regulerende functie van corticosteroiden op de malaria-immuniteit in het malaria model van de muis krijgt extra aandacht, omdat deze resultaten het uitgangspunt vormden voor het onderzoek zoals beschreven in dit proefschrift.

HOOFDSTUK II beschrijft het gebied waar het onderzoek plaats vond en de wijze waarop de diverse onderzoeksgroepen werden samengesteld. Een korte beschouwing over de diverse termen en begrippen zoals gehanteerd in dit proefschrift wordt gegeven. De manier waarop de verschillende parameters werden gemeten tijdens het veldonderzoek in Tanzania en daarna op het laboratorium voor chemische en experimentele endocrinologie (hoofd prof. dr. Th. J. Benraad) van het St. Radboud Ziekenhuis te Nijmegen worden besproken. Er wordt daarbij uitgebreid ingegaan op de laboratorium-bepalingen zoals die gebruikt werden voor het meten van het totale cortisol gehalte in het serum. Er werd daarbij vooral gelet op de interassay variabiliteit gezien de vele assays die nodig waren voor het meten van een dergelijk groot aantal monsters. De mogelijke invloed van langdurige anti-malaria profylaxe op een verlies van immuniteit wordt besproken.

HOOFDSTUK III geeft de resultaten weer van twee malaria surveys waarbij met behulp van de frequentie van een palpabele milt en het vinden van parasieten in het bloed vastgesteld werd, dat het hier een hyperendemisch malaria gebied betrof. De seizoensinvloeden werden aangegeven met behulp van malariologische en klimatologische gegevens.

HOOFDSTUK IV beschrijft de frequentie van parasitaemie en palpabele milt bij een referentie groep van gezonde

niet-zwangere vrouwen. Tevens werd de concentratie van het totale cortisol en de fractie vrij cortisol bepaald in het serum van deze vrouwen. Er werd een aanwijzing gevonden voor een geringe afname van de concentratie van het totale cortisol gehalte met stijgende leeftijd. Alhoewel de concentratie van het totale cortisol in parasiet-positieve vrouwen niet verschilde van die van parasiet-negatieve vrouwen, liet het kleine aantal parasiet-positieve vrouwen geen verdere conclusies toe. Hetzelfde gold ook voor de waargenomen hogere fractie van het vrije cortisol bij vrouwen met parasitaemie.

HOOFDSTUK V beschrijft de resultaten van de transversale studie bij zwangere vrouwen, waarbij niet het voorkomen van klinische malaria maar parasitaemie werd bestudeerd. Parasitaemie werd vaker gevonden in nulliparae dan in multiparae; bovendien nam binnen de multiparae de frequentie af met het stijgen van de pariteit. Er werd geen duidelijke relatie tussen parasitaemie en amenorrhoeduur gevonden. Het voorkomen van een vergrote milt correleerde wel met parasitaemie. Werd koorts als parameter genomen, dan werd deze correlatie niet gevonden. Dit resultaat was te verwachten aangezien niet de klinische malaria (parasitaemie + symptomen), maar slechts de aanwezigheid van parasieten werd bestudeerd. De concentratie van het totale cortisolgehalte bleek hoger te zijn in nulliparae dan in multiparae; tevens vertoonden de parasiet-positieve vrouwen een hogere concentratie dan de parasiet-negatieve vrouwen. De gemeten verschillen waren echter niet voor alle zwangerschapsperioden significant. Er werd geen relatie gevonden tussen de hoogte van de parasitaemie en de cortisolconcentratie.

HOOFDSTUK VI geeft de resultaten weer van de longitudinale studie van zwangere vrouwen, waarbij niet het vinden van parasieten in het perifere bloed, maar het voorkomen van klinische malaria als maatstaf werd genomen voor het verlies van malaria-immuniteit. Klinische malaria kwam vaker voor bij nulli- dan multiparae; de leeftijd speelde geen rol bij

nulliparae, maar een geringe afname met stijgende leeftijd werd gevonden binnen de groep van multiparae. De frequentie van klinische malaria was afhankelijk van de amenorrhoeduur: klinische malaria kwam vaker voor in de periode tussen de 12° en 18° zwangerschapsweek. De parasietendichtheid in geval van klinische malaria werd niet beinvloed door pariteit of zwangerschapsduur. Het voorkomen van een vergrote milt of koorts werd wel beinvloed door klinische malaria hoewel het vinden van een vergrote milt nadelig beinvloed werd door een toenemende amenorrhoe duur. Het haemoglobine gehalte (Hb) tijdens zwangerschap was afhankelijk van de zwangerschapsduur, pariteit en klinische malaria. Nulliparae en/of vrouwen met klinische malaria hadden de laagste Hb concentraties, waarbij een amenorrhoe van 16 tot 26 weken gepaard ging met lagere Hb waarden. Het geboortegewicht van kinderen was hoger bij multiparae dan primiparae. Het voorkomen van klinische malaria tijdens de zwangerschap had slechts een geringe en niet significante invloed op het geboortegewicht. Prematuriteit van een kind werd in deze studie bepaald aan de hand van het geboortegewicht van het kind. De frequentie van prematuriteit was niet gebonden aan pariteit noch aan het voorkomen van klinische malaria. Dezelfde bevindingen kwamen naar voren met betrekking tot de conditie van het kind bij de geboorte, zoals die gemeten werd met behulp van de Apgar score. De serum concentratie van het totale cortisol nam significant toe tijdens zwangerschap met eveneens significante verschillen tussen nulliparae en multiparae. Nulliparae hadden hogere serumconcentraties van totaal cortisol, onafhankelijk van leeftijd en zwangerschapsduur. Vrouwen met klinische malaria hadden hogere cortisolspiegels tijdens de aanval en daarna in vergelijking met vrouwen zonder klinische malaria, waarbij het eerder genoemde verschil tussen nulli- en multiparae bleef bestaan. Er waren aanwijzingen dat deze vrouwen met malaria reeds vóór de malaria doorbraak hogere cortisol spiegels hadden. De concentratie van het vrije cortisol, indirect bepaald middels de fractie vrij cortisol, vertoonde dezelfde veranderingen als de concentratie van het totale

cortisol, waarbij het pariteits effect sterker was dan de invloed van malaria.

HOOFDSTUK VII bevat de gegevens van het onderzoek tijdens en na de bevalling. Parasitaemie werd bepaald in perifeer moederlijk bloed, in de placenta en in het bloed van de vena umbilicus. In de placentae werd een hogere parasieten dichtheid gemeten dan perifeer, waarbij de hogere parasieten dichtheid van primiparae vergeleken met multiparae gepaard ging met het vaker voorkomen van congenitale parasitaemie. De grootte van de milt werd bepaald na de geboorte van het kind en bleek vaker vergroot dan op grond van cijfers tijdens de zwangerschap werd vermoed. Vergelijking van deze bevindingen met die uit andere studiegroepen toonde aan dat het bepalen van de miltgrootte tijdens zwangerschap een onbetrouwbare methode is om malaria prevalentie te bepalen. Histologische veranderingen in de placenta in relatie tot malaria gaven sterke aanwijzingen voor passage van parasieten en derhalve tot het optreden van congenitale parasitaemie. Kinderen van moeders met een malaria infectie in de placenta hadden een lager geboortegewicht, maar het verschil was niet significant. De invloed van de pariteit was echter wel weer significant. In deze studie werd geen aanwijzing gevonden voor het vaker prematuur geboren worden van kinderen in de groep vrouwen met een malaria infectie van de placenta, terwijl de conditie van het kind bij de geboorte evenmin hierdoor beinvloed werd.
De baring ging gepaard met extreem hoge cortisol concentraties, waarbij het pariteitsverschil tussen primiparae en multiparae behouden bleef. Een gecompliceerde bevalling had een additionele stress tot gevolg bij de moeder met als gevolg extra hoge cortisolwaarden. Parasitaemie bij de moeder of in de placenta had geen invloed op de cortisolwaarden van moeder noch kind. Totale cortisolwaarden van het kind werden eveneens niet hoger gevonden in geval de moeder een primipara was, ondanks de hogere cortisolwaarde bij deze moeders. De additionele stress van een abnormale bevalling ging gepaard met een hogere cortisol concentratie bij de

neonaat, wat zou kunnen berusten op een toegenomen passage van het vrije cortisol door de placenta of op een stimulatie van de hypofyse-bijnier als bij het kind.
Totale cortisolwaarden normaliseerden in 3 à 4 dagen post-partum met een relatief snellere daling bij vrouwen met een abnormale bevalling, hetgeen erop zou kunnen wijzen dat het hier een hogere vrije cortisolconcentratie betrof in deze moeders tijdens de baring.

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# S T E L L I N G E N

behorende bij het proefschrift

CORTISOL AND MALARIA IMMUNITY IN HUMAN PREGNANCY

M.P.H.Vleugels

15 november 1984

I De verhoogde frequentie van klinische malaria gedurende de eerste helft van de zwangerschap en met name de daarop volgende afname kunnen wijzen op een aanpassing van de immuunreactie die minder gevoelig wordt voor immunosuppressie.

(dit proefschrift)
(Brabin B.J. (1983), Bull. WHO, 61 p. 1005 )

II Dat de morbiditeit van een malaria infectie pariteits onafhankelijk is behoeft niet in tegenspraak te zijn met een veronderstelde verbeterde malaria immuniteit in multiparae ter verklaring van een lagere frequentie van malaria in deze groep ten opzichte van nulliparae.

(dit proefschrift)

III In een hyperendemisch malaria gebied met een "primary health care", hoeft malaria tijdens zwangerschap niet tot lagere geboortegewichten noch tot het vaker voorkomen van partus prematurus te leiden.

(dit proefschrift)

IV De significant hogere cortisol concentratie tijdens zwangerschap en partus bij nulliparae vergeleken bij multiparae, kan wijzen op een pariteits afhankelijk verschil in immuno-reactiviteit.

(dit proefschrift)

V De relatie tussen de ernst van een placentaire infectie en het voorkomen van defecten in de syncytiotrophoblast en de basaalmembraan geeft aan dat de defecten een belangrijke oorzaak voor congenitale malaria kunnen zijn.

(dit proefschrift)

VI "The greatest disadvantage a person experiences may come not from lack of wealth but from lack of knowledge; not from poverty but from ignorance."

(Julius K. Nyerere, president of the United republic of Tanzania: Maua, 1982)

VII Het economisch en sociaal belang van uitzending van jonge tropenartsen wordt nog onderschat door belangenorganisaties, die gemoeid zijn met het probleem van de overbelaste arbeidsmarkt.

VIII "Alleen een, op onderlinge waardering en respect gebaseerde samenwerking tussen eerste en tweede echelon kan verhinderen dat hooggekwalificeerde kennis wordt misbruikt om de fysiologie van het voortplantingsproces te perverteren."

(Kloosterman, G. J. prof. dr. (1984), Med. Contact )

IX De betekenis van een persisterend "nicotine abusus" tijdens zwangerschap ligt niet alleen in een mogelijk nadelige invloed op de foetale groei, maar is ook vaak een symptoom van een levenswijze gekenmerkt door abusus van andere, voor de foetale ontwikkeling nadelige genotsmiddelen.

(Jerushalmy, J. (1970), Am. Jrn. Epidemiology, 93, p. 443)

X Indien het vermoeden rijst op tuberculose bij een ondervoed kind in een ontwikkelingsland, is er geen plaats meer voor twijfel maar moet behandeling onverwijld gestart worden.

XI Ook het verrichten van wetenschappelijk cq. promotie onderzoek dient binnen het cluster-verband van universitaire en perifere opleidingen een integraal onderdeel te zijn van de vorming tot gynaecoloog.

XII De term "Verloskunde en Roken", geeft beter weer dan "Roken en zwangerschap", dat de medeverantwoordelijkheid voor perinatale morbiditeit t.g.v. "nicotine abusus" ook ligt bij de begeleider van de zwangere, en reeds begint voor de conceptie.

(Donovan, J.W. (1977), Brit. Jrn. Prev. and Social Med. 31, p. 6)

XIII De huidskleur van de thans in Africa gebruikte pleisters, passen meer bij de huidskleur van de tropenarts dan van de patient zelf waarvoor ze bedoeld zijn.